



Review Article

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Proven Activities of *Entada phaseoloides* (L.) Merr.

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Abstract

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Chemical compounds in plants mediate their effect: on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. *Entada phaseoloides*, a well-known liana widely used therapeutically and has become increasingly popular as an important medicinal plant. *Entada phaseoloides* and its bioactive compounds possess various pharmacological properties. The plant has been traditionally used in Ayurvedic medicine for centuries as an anti-inflammatory, analgesic, antipyretic, antiarthritis, antidiabetic, antioxidant, cytotoxic, hepatoprotective, antimicrobial and molluscidal agent. The present review summarizes current knowledge on reported medicinal properties, pharmacological actions. Despite this, further investigations are required to explore *Entada phaseoloides* and to evaluate the different biological activities of either its extracts or the isolated compounds with probable modes of action.

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Introduction

The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80% of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more in recent years as scientific evidence

about the effectiveness of herbal medicine has become more widely available.

In 2001, researchers identified 122 compounds used in modern medicine which were derived from ethno medical plant sources (Achrekar et al., 1991), 80% of these compounds have had an ethno medical use identical to the current use of the active synthetic drugs (Patil et al., 2009). Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine and opium. The use of herbs to treat disease is

almost universal among non-industrialized societies (Ananthanarayanan and Panikar, 1992; Augusti, 1975) and is often more affordable than purchasing expensive modern pharmaceuticals. Researchers found that people in different parts of the world tended to use medicinal plants for medical purposes. In the early 19th century, when chemical analysis first became available (Bhadauria, 2002; Cherian and Augusti, 1993), scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favour of drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Lai and Roy, 2004). Most plant-derived extracts are generally recognized as safe (GRAS) (21 Code of Federal Regulations (CFR) × 182, 184) (Burt, 2004).

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions (Mahalingam and Krishnan, 2008) and to defend against attack from predators such as insects, fungi and herbivorous mammals. One of such widely used medicinal plant is *Entada phaseoloides*.

Entada phaseoloides

This perennial climbing vine is well known for its enormously large seeds and seed pods, which can grow well over 5 feet (1.5 meters) in length, and contain a dozen or more seeds per seed pod. The seeds have a very hard woody shell. These seeds are dark brown to black in color and are shaped like round disc, measuring approximately 2 inches by 2 inches (5 cm × 5 cm). This vine primarily grows in tropical zones at or near sea level along beaches, coastlines and along the sides of rivers, throughout the countries on Indian Ocean: Madagascar, Southern Africa, Asia, and Australia. Because the seed coat is thick, waxy and airtight, the seeds often travel through waterways for years before they are able to take root; once rooted they quickly encircle surrounding trees and grow skyward. When these vines are left to grow wildly, their base can grow as thick as a small tree, easily obtaining a diameter of 8 – 12 inches (0.2 – 0.3 meters) or more. The leaves grow in pairs along the length of the limbs and are dark green with a glossy sheen.

This comprehensive review summarizes our current knowledge of the major bioactivities and clinical efficacy of *Entada phaseoloides* as one of the currently used popular herbal plant.

Entada as tribal medicine

Dried vine materials of about 15 g to 30 g in decoction are reported to be effective in rheumatic lumbar, leg pains, sprains and bruise. Powdered seeds when taken orally with water can cure jaundice and edema due to malnutrition. In abdominal pains and colic, the kernels of seeds after mixing with oil can be applied as paste onto affected area. The paste of seed is used for reducing irritation and applied to glandular swellings in the joints, and swollen hands and feet. It is also used as hair growth stimulant. Seeds are used as emetic. It is used as antipyretic agent. In South Africa, seeds are used by infants to bite on during their teething period. Also, used as remedy for cerebral hemorrhage. As a treatment of skin itches, the affected part is washed with a decoction of the bark of *Entada phaseoloides*. Stem macerated in cold water used as cleansing soap and also used as an emetic.

Review of proven medicinal properties

1) Anti-inflammatory and analgesic activities

On the basis of various previous study models, saponins obtained from *Entada phaseoloides*, were reported to have significant anti-inflammatory activity and specifically the saponin from seed kernels of *Entada phaseoloides*, was reported to have significant activity against Walker 256 carcinosarcoma in rats (Liu et al., 1972).

Anti-inflammatory activity of saponin was proven in the study of methanolic extract of *Entada phaseoloides* seeds in animal models where the LD50 was found to be more than 5,000 mg/kg in acute oral toxicity testing. Pre-treatment with the extract (400 mg/kg) reduced carrageenan induced rat paw edema at 3rd hour compared to control group of rats. Dose dependent (100, 200 and 400 mg/kg) reduction in inhibition of granuloma formation of cotton pellet granuloma, exudate volume, and total leukocyte count was observed with the extract. The extract inhibited acetic acid induced writhing dose dependency (40, 80 and 120 mg/kg) but was found inactive in reducing the pain produced by thermal injury. C-reactive proteins were

absent in extract treated group. The results indicated that extract possesses weaker acute but strong sub-acute anti-inflammatory activity and strong peripheral analgesic activity. The results also suggested that the extract may act on the “proliferative phases of inflammation” (Gupta et al., 2006).

In another study of anti-inflammatory effect of topical application of different formulations of seed pulp of *Entada phaseoloides* was evaluated. After removing the shell, *Entada phaseoloides* seeds were powdered. Paste was prepared with water and ointment with polyethylene glycol and Carbowax 3350. 32 Wistar rats of either sex weighing 140-200 g were divided into four groups, Group-I vehicle, Group-II *Entada phaseoloides* paste, Group-III *Entada phaseoloides* Ointment, Group-IV Diclofenac sodium Ointment. Arthritis was induced by injecting 0.1ml Complete Freund's adjuvant in sub plantar region of the left hind paw. Drug treatment was started on the same day and given for 12 days. Paw volume was measured with Plethysmometer on day 0, 1, 5, 12 and 21 for both the paws. Bodyweight and Gait was observed throughout the study. Localized inflammatory reaction was developed in all the rats in 24 h. In control group, there was no resolution of swelling even in 21 days. Both EP formulations showed significant ($p < 0.001$) anti-inflammatory activity as compared to that of control. *Entada phaseoloides* ointment was equi-effective to that of diclofenac sodium on 12th day. Its paste was significantly ($p < 0.05$) found to be more effective than diclofenac sodium on 21st day. Both the formulations of *Entada phaseoloides* were found to have anti-inflammatory activity, but the paste was significantly more effective than Diclofenac sodium (Dawane et al., 2011).

Inflammatory response in the CNS mediated by microglia cells play an important role in host defense and is implicated in the pathology of neurodegenerative diseases. Owona et al. (2013) investigated the capacity of *Entada africana* to protect microglia from inflammatory insults by exploring the effect of the $\text{CH}_2\text{Cl}_2/\text{MEOH}$ 5% fraction (Ea5) on pro-inflammatory cytokines mRNA expression. Finally, we studied the effect of Ea5 on the inhibition of p38 MAPK Kinase. The results were compared to those obtained with Baicalin, a well reported anti-inflammatory flavonoid.

Authors found that Ea5, as well as Baicalin inhibited LPS-induced NO production in a dose dependent manner. Ea5 was most active in term of NO inhibition

(87.07%), in comparison to Baicalin (70.85%). The expression of $\text{TNF}\alpha$, IL-1 β , IL-6 and iNOS was strongly suppressed by Ea5 in microglia. Ea5 also inhibited the activity of p38MAPK Kinase, up to 30% for the concentrations tested, whereas a prominent inhibition was obtained with Baicalin.

2) Anti arthritic activity

The effect of two formulations of *Entada phaseoloides* seeds after topical application in “monoiodoacetate-induced osteoarthritis” in rats was studied since arthritis is a very common clinical condition affecting both sexes and all ages. Most common forms of arthritis are osteoarthritis and rheumatoid arthritis. In all types of arthritis pain, inflammation and functional restriction are the presenting manifestation. Anti-inflammatory drugs like NSAIDs, corticosteroids and disease-modifying antirheumatic drugs, etc. are used for symptomatic relief, but many times they are associated with adverse effects that can often be as difficult to manage as the disease itself. Therefore, a need exists for new ways to treat these patients.

The effect of topical application of two formulations of *Entada phaseoloides* (EP) seeds was studied in the MIA (monoiodoacetate-induced osteoarthritis) model in rats. Both the paste and ointment formulations of EP were tested on 32 Wistar rats weighing 150-200 g, divided into four groups as (I) vehicle, (II) EP paste, (III) EP ointment and (IV) diclofenac ointment. Osteoarthritis was induced by intra articular injection of 50 μL of MIA solution.

Drug treatment was given topically according to groups for 14 days. Animals were observed for joint inflammation and gait. Joint histopathology was studied and scored. Swelling and redness of left knee was seen in all rats within 24 hrs which subsided gradually. Lameness to gait and thickening of the joint capsule was seen only in control rats. Histopathologically, osteoarthritic changes were significantly less in drug-treated groups compared to control. As a result, both the formulations of EP were found to be effective in preventing the damage to the joint (Dawane et al., 2013).

3) Antidiabetic and hypolipidemic activities

On the basis of previous study, Zheng and his associates (2012a,b) reported that the TSEP (Total Saponin from *Entada phaseoloides*) dramatically reduce the fasting blood glucose and serum insulin levels and alleviated

hyperglycemia associated with oxidative stress in T2DM (type 2 Diabetes mellitus) rats. Moreover, a significantly hypolipidemic effect and an improvement in tissue steatosis were observed after TSEP administration. Further investigations revealed a possible anti-inflammation effect of TSEP by examining serum levels of IL-6 (interleukin-6), TNF- α (tumor necrosis factor- α) and CRP (C-reactive protein). The effects of TSEP exhibited a dose-dependent manner and were comparable to metformin. Both hypoglycemic and hypolipidemic activities of TSEP in T2DM rats supported its anti-diabetic property. TSEP exerted its therapeutic effect through repressing chronic inflammation responses.

In another experimental model, the anti-diabetic effects of ethyl acetate, petroleum ether and chloroform fractions were investigated from the methanolic extract of seeds of *Entada phaseoloides* in AIDM (alloxan induced diabetic mice) by Ikram and his associates (2011). The effect of these fractions (200 mg/kg body weight i.p) was observed on FBG (fasting blood glucose) level and active fraction was further investigated for its dose dependent activity (250 mg/kg and 350 mg/kg body weight) on fasting blood glucose level and also on TC (total cholesterol), TG (triglyceride), SGOT (serum glutamate oxaloacetate transaminases) and SGPT (serum glutamate pyruvate transaminases) level in AIDM which showed significant effects. The most significant reduction of FBG level of around 72.02% was observed for Et-Ac fraction in AIDM. A significant reduction ($p < 0.05$) in serum TC and TG level of 53.00% and 57.25% respectively was also found for Et-Ac fraction of *E. Phaseoloides*. The hypoglycemic and hypolipidemic activities were comparable to metformin HCl (150 mg/kg). In diabetic mice, SGOT and SGPT levels were significantly elevated that were further reduced after intra peritoneal administration of this fraction. These results indicated ethanolic fraction of *E. Phaseoloides* have favorable effects in bringing down the severity of diabetes together with hepatoprotectivity.

An another study was carried out by Zheng and others (2012a,b) to observe the effect of TSEP (total saponins from *Entada phaseoloides*) on islet morphology and skeletal muscle PI3K pathway-related protein expression of type 2 diabetic rats, the type 2 diabetic rats were induced by high-fat diet and low-dose streptozotocin and then randomly divided into 5 groups, i.e., the normal control, the model group, the positive control drug (200

mg·kg⁻¹ metformin), the low-dose TSEP (25 mg·kg⁻¹) group and the high-dose TSEP (50 mg·kg⁻¹). Three weeks later, the islet morphology of rat pancreas were observed by HE (Hematoxylin and Eosin) staining, and protein expressions of IRS-1 (insulin receptor substrate-1), PI3K (phosphatidyl inositol 3-kinase), PTP-1 B (protein tyrosine phosphatase-1B) and GLUT4 (glucose transporter 4) in rat skeletal muscle were detected by Western blot. When compared with the model group, TSEP administered groups showed relatively normal structures, clear pancreatic cells and intact capsula structures in pancreatic tissue pathological sections, with the number of pancreatic islets close to the normal control group. Meanwhile, above TSEP administered groups showed increased IRS-1, PI3K and GLUT4 protein expressions in their skeletal muscle tissues and decreased PTP-1B protein expression compared with the model group. TSEP has an effect on protecting pancreatic tissues of type 2 diabetic rats and intervening in abnormal expression of proteins in skeletal muscle tissues.

4) Anti-toxicity activities

Xiao and associates (2007) studied the impact of the crude and processed products of *Entada phaseoloides* on gastrointestinal movement in mice with the methods of charcoal propulsion of small intestine and methyl orange colorimetry of gastric emptying to observe acute-toxicity. The oral LD50 of crude *Entada phaseoloides*, and two processed products of *Entada phaseoloides* in mice were 27.17 g/kg, 35.13 g/kg and 42.18 g/kg body weight respectively. Crude and processed products of *Entada phaseoloides* can significantly promote the enteric propulsion of normal mice, and can significantly counteract the depressing status induced by atropine, but have no influence on the overactive status induced by neostigmine. The high, middle and low-dose of groups showed significant inhibition of the gastric emptying in normal mice. Processed *Entada phaseoloides* showed effects on the enteric propulsion of normal and depressing mice, can restrain the gastric emptying under normal mice, but its safety was better than crude *Entada phaseoloides*.

5) Antiulcer activities

The ethanol extract of the seeds of *Entada phaseoloides* was assessed for its antiulcer activity against aspirin plus pylorus ligation induced gastric ulcers in rats by Ramakrishna and others (2008). HCl- ethanol induced ulcer in mice and water immersion stress-induced ulcers in rats. A significant ($p < 0.001$) antiulcer activity was

observed in all the models. The parameters taken to assess antiulcer activity were volume of gastric secretion, free acidity, total acidity and ulcer index. From the screening results it was observed that, in aspirin pylorus ligation induced gastric ulcer method, the ethanol extract of *Entada phaseoloides* showed moderate reduction in gastric volume, free acidity and ulcer score as compared to control.

The results also showed that in HCl-induced lesion in mice and cold restraint ulcer in mice, administration of ethanolic extract of *Entada phaseoloides* inhibited ulcer considerably when compared with the standard. They concluded that the seeds of *Entada phaseoloides* contain chemical constituents entadamide A, B and C, phaseoloides and most likely these active constituents were responsible for antiulcer activity.

6) Hepatoprotective activities

Gupta and others in 2011 conducted the study to investigate the protective activity of alcoholic extract prepared from the stem of *Entada pursaetha* (PSE) syn. *E. phaseoloides* against carbon tetrachloride (CCl₄)-induced hepatotoxicity in male Wistar rats. The animals were divided into six groups (groups I to VI) with six animals in each and different treatments were given for seven days. Groups I and II were naïve and vehicle control groups and were given NSS and 2% polysorbate 80 aq. solutions, respectively. In group III, hepatoprotective drug, silymarin, was given orally @ 50 mg/kg bwt as standard control. In groups IV to VI, hepatoprotective effect of PSE was determined at three different oral doses of 30, 100 and 300 mg/kg bwt, respectively. On 7th day, three hour after the last administration, CCl₄ intoxication (@2ml/kg bwt, 1:1 v/v in olive oil, i.p.) was used to induce acute hepatotoxicity in all groups except group I. Animals of all groups were sacrificed 24hour after the CCl₄administration. Liver weight, relative liver weight, alkaline phosphatase (ALP) and creatinine levels were determined in different groups. The PSE @ 100 and 300 mg/kg b. wt. resulted in a significant dose dependent hepatoprotective effect comparable to standard control, by preventing increase in liver weight and relative liver weight. In addition, serum ALP level in rats given PSE at all the three doses was significantly lower than vehicle control rats. There was no significant effect of PSE on serum creatinine at any of the three dose levels in comparison to vehicle control group. Thus study reveals that alcoholic extract of *E. pursaetha* has significant hepatoprotective activity

similar to standard drug, silymarin, in CCl₄ -induced acute hepatotoxicity in rats.

7) Anticomplement and antimicrobial activities

Seventeen flavonoids isolated from the extracts of the stem of *Entada phaseoloides*, were investigated by Li and associates (2012) for their anticomplement (both classic and alternative pathways) and antimicrobial activities against Gram-positive bacteria MSSA (Methicillin sensitive *Staphylococcus aureus*), MRSA (Methicillin resistant *Staphylococcus aureus*), Standard *Enterococcus* and *Bacillus subtilis*], Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and the yeast-like pathogenic fungus *Candida albicans*. The anti-complement studies revealed a dose-dependent activity among isolated quercetin, luteolin, apigenin, galangin, 5,2',5'-trihydroxy-3,7,4'-trimethoxyflavone-2'-O-β-D-glucoside(+)-3,3',5',5,7-pentahydroflavanone, (+)-dihydrokaempferol, (-)-epicatechin, (+)-catechin, naringenin, and 5,7,3',5'-tetrahydroxyflavanone, and the antimicrobial results indicated that quercetin, 5,7,4'-trihydroxy-3'-methoxyflavonol and galangin produced the inhibitory activities against MRSA, MSSA, and Standard *Enterococcus*, while luteolin and rhamnocitrin displayed inhibition against only MRSA and MSSA.

8) Antioxidant and cytotoxic activities

Yasuraokaet and others (1977) reported that the bark of *Entada phaseoloides* exhibits potent molluscidal activity against *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* with LC₅₀ of 3.6-5.8 ppm since *Entada phaseoloides* remained stable over a wide range of pH values, in the presence of minerals and yeast cells and after ultraviolet irradiation of solutions.

Garcia and associates (1981) under field conditions, proved that the *Entada phaseoloides* bark at a dose rate of ≥ 40 g/m² was sufficient to produce a satisfactory molluscidal effect or commercial *Entada phaseoloides* bark at a dose rate of 2 g/L of water with *Oncomelania quadrasi* can kill 100% of snail within 24 h. Thus the molluscidal effect of *Entada phaseoloides* is very much effective in controlling Schistosomiasis, a snail transmitting debilitating and fatal endemic disease which is of major public health concern.

The methanolic crude extract of the bark and seed of *Entada phaseoloides* (L.) Merrill and its different organic soluble partitionates were screened for

antioxidant, cytotoxic, membrane stabilizing and antimicrobial activities by Aktar and others (2011). The crude extract, carbon tetrachloride and aqueous soluble fractions of both bark and seed showed higher level of total phenolic content (TPC, 245.59, 240.22, 240.03 and 117.0 mg of Gallic acid equivalent (GAE)/gm of dried extract). In the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, the crude extract of bark and its chloroform and aqueous soluble fractions demonstrated strong antioxidant property with the IC₅₀ of 3.24, 1.55 and 3.6 µg/mL, respectively, whereas, all the fractions of seed extract revealed mild antioxidant activity. The petroleum ether soluble fraction of both seed and bark exhibited significant cytotoxicity (LC₅₀ = 1.54 mg/mL and 5.4 mg/mL) which confers the presence of bioactive metabolites in this plant. On the other hand, the crude extract of seed and petroleum ether soluble fraction of bark inhibited the hemolysis of RBC of rat's blood by 78.89% and 57.43%, respectively, as compared to 84.44% exerted by acetyl salicylic acid (0.10 mg/mL). In antimicrobial screening, the carbon tetrachloride soluble fraction of bark showed significant antimicrobial activity against *Staphylococcus aureus* (zone of inhibition = 17.0 mm) with MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values of 7.81 mg/mL and 125 mg/mL, respectively.

Entada africana, a widely used African medicinal plant of the same genus *Entada*, has been reported for various medicinal properties. When the acute toxicity of the methanolic stem bark and leaf extracts of *Entada africana* Guill. and Perr., (Mimosaceae) was assessed on mice, it revealed an average toxicity with a LD₅₀ of 146.7 mg·kg⁻¹ and 249.9 mg·kg⁻¹ body weight for stem barks and leaves, respectively. The extracts showed no cytotoxicity against Human epidermoid carcinoma (KB) and African green monkey (Vero) cells. Sub-chronic toxicity was assessed in rabbits, which received orally, daily for a month, a dose corresponding to 10% of the LD₅₀. Compared to the control group, this dose caused no significant ($p > 0.05$) modification of haematological and biochemical parameters, total cholesterol, urea, creatinine and AST (aspartate amino-transferase). The extracts lowered serum glucose significantly ($p < 0.05$) by 52% at first two weeks of treatment. The stem bark and leaf extracts showed temporary decrease ($p < 0.05$) of ALT (alanine amino transferase) by 26.1 % and 39.1%, respectively. The stem bark extracts increased triglycerides significantly ($p < 0.01$) by 108% at the end of last week of treatment. These investigations seemed

to indicate the safety of sub-chronic oral administration (up to 14.67 mg·kg⁻¹ and 24.9 mg·kg⁻¹ body weight) of the methanolic extracts of *Entada africana* in rabbits (Tibiri et.al, 2007).

Conclusion

Entada phaseoloides offers a wide range of ethnobotanical utilizations, which are based on the diverse patterns of secondary metabolites of which most abundant are saponins, diterpenes, triterpenes, flavanoids and phenolic compounds. Phenolic compounds, saponins, diterpenes as well as triterpenes are responsible for the pharmacological properties of *Entada phaseoloides* such as anti-inflammatory, antidiabetic, antitumour and analgesic activity (saponin); anticomplement, antimicrobial and antioxidants (phenolics); antiulcer (entadamides), antitoxic, hepato protective (triterpenoid glycosides) in addition to molluscicidal activities (saponin).

Many of these phytochemicals have beneficial effects on long - term health when consumed by humans, and can be used to effectively treat human diseases. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work (Manoj et al., 2008) This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

Bark, leaves and stems of *E. scandens* viz., *phaseoloides* shows activities like antitoxic, antipyretic, alexeteric, anthelmintic, antiperiodic, antispasmodic, antitussive, astringent, carminative, emetic, febrifuge, narcotic and tonic. (www.herpathy.com). These properties are also exhibited by seeds. In most of the pharmacological experiments, seed extract of the plant has been used. With the use of seed extract continuation and survival of this medicinally important liana is threatened. So instead of using seed extract, if extract of bark, leaves and stems is used for animal experiments to prove their medicinal activities which are same as that of seeds, this endangered plant will be protected from exploitation which is the need of the hour.

Another species of *Entada* i.e., *E. africana* has same phytoconstituents and possesses same medicinal properties as that of *E. phaseoloides* The extracts of the

plant showed no cytotoxicity against Human epidermoid carcinoma (KB) and African green monkey (Vero) cells. (Tibiri et al., 2007). Using the *E. phaseoloides* extracts the same line of work can be pursued.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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