ABSTRACT

Plants are a great source of medicines, especially in traditional medicine, which are useful in the treatment of various diseases. *Tylophora indica* is a climbing perennial plant indigenous to India. It grows wild in the southern and eastern region. It has long-standing reputation as a remedy for asthma. The leaves of *Tylophora indica* are included in Bengal pharmacopeia since 1884. It is said to have laxative, expectorant, diaphoretic (Sweating) and purgative (Vomiting) properties. It has been used for the treatment of various respiratory problems. It has reputation as an alternative and as a blood purifier, often used in rheumatism and syphilitic rheumatism. Root or leaf powder is used in diarrhea, dysentery and intermittent fever. It is an expectorant and administered in respiratory infections, bronchitis and whooping cough. Dried leaves are emetic, diaphoretic and expectorant. It is regarded as one of the best indigenous substitute for ipecacuanha, so it was considered as Indian ipecacuahna in the latter half of the 19th century. Other problems including allergies, bronchitis and colds, as well as dysentery and osteoarthritis pain. Tylophora has increasingly popular for treatment in asthma, based on its traditional use for this purpose. *Tylophora indica* is also still recommended for some of its other traditional uses, including hay fever, bronchitis and the common cold.

**Keywords:** Antiasthmatic , Asclepiadaceae , Herbal remedies ,Indian ipecacuahna , Tylophora indica, Tylophorine.

INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The widespread use of herbal remedies and health care preparations, as described in ancient texts including the Vedas, holy Koran and the Bible are obtained from commonly used traditional herbs and medicinal plants. In India, approximately 1700 plant species are used in Ayurveda, 500 for Siddha, 400 for Unani, 300 for Amchi systems of medicine with substantial overlaps of common plants among these systems. The trend of using natural products is increasing steadily. The use of traditional medicines and medicinal plants in most developing countries as a normative basis for maintenance of good health has been widely observed. Further an increasing reliance on the use of medicinal plants in the industrialized societies has been related to
the development of several drugs and chemotherapeutics from plant species as well as from traditionally used rural herbal preparations. Herbal remedies have attained much more popularity in the treatment of minor ailments, due to increasing awareness of personal health maintenance through natural products. Indeed, the market and public demand has been so great that there is a great extinction risk to many medicinal plants and obviously the loss of genetic diversity.

Tylophora indica (Burm f.) Merill. (Family: Asclepidaceae) commonly known as Antmul is a twining perennial plant distributed throughout southern and eastern part of India in plains, forests, and hilly places. The plant is found growing normally in Uttar Pradesh, Bengal, Assam, Orissa, Himalayas and sub Himalayas in India. It is a branching climber or shrub that grows up to 1.5 meters, leaves are obvate-oblong to elliptic-oblong, 3-10cm long and 1.5-7cm wide. Roots Long fleshy with longitudinally fissured light brown, corky bark. Flowers minute, 1-1.5 cm across, in 2-3 flowered fascicles in axillary umbellate cymes. Calyx divided nearly to the base, densely hairy outside; segments lanceolate, acute. Corolla greenish yellow or greenish purple; lobes oblong, acute. Fruit a follicle, up to 7 × 1cm, ovoid lanceolate, tapering at apex forming fine mucro, finally striate, glabrous. Seeds 0.6-0.8 × 0.3-0.4cm long. The plant has been reported to contain 0.2-0.46% alkaloids viz. Tylophorine, tylophorinine, tylophorinidine, (+)septicine, isotylocrebrine, tylophorinicine, sterols, flavanoids, wax, resins, and tannins. The plant has been traditionally used for the treatment of bronchial asthma, jaundice and inflammation. Its antitumor, immunomodulatory, antioxidant, antiasthmatic, smooth muscle relaxant, antihistaminic, hypotensive, antirheumatic activities are scientifically proven. In Ayurveda, the plant has been used in treatment of asthma, dermatitis and rheumatism. Although the leaf and root of this plant are widely used for treating jaundice in Northern Karnataka, there is a paucity of scientific evidence regarding its usage in liver disorder. The other reported activities include immunomodulatory activity, anti-inflammatory activity, anticancer activity and antiamoebic activity.
Botanical name: Tylophora indica (Burm.f.) Merrill.
Synonym: Tylophora asthmatica (Linn.F).
Common Name: Antmul.
Other names:
Beng,- Antomul.
Bomb.- Pitmari, Kharaki-raena, Anthamul, Pitakari.
Guj.- Antamul.
Hindi- Antamuli.

Figure 1
Tylophora indica (Burm f.) Merrill.
It is Indigenous to India. The plant inhabits up to an elevation of 1,260m in the sub Himalayan tract and in central and in peninsular India. It also met within Eastern, North-East and Central India, Bengal and parts of South India.[4].

Habitat

The plant is a perennial branching climber with long fleshy roots. It grows in planes and hilly places of India up to an attitude of 1,000 m in Bengal, Assam, Cachar, Orissa, and southern India[11]. Found in the plains, forests, and hilly slopes and outskirts of the forest. Forms dense patches in the forest in moist and humid conditions in open hill slopes and narrow valleys, also cultivated for its medicinal uses. The plant shows stunted growth in the areas with lesser rainfall.

Botanical Description

a) Macroscopy

Leaf 5-10 cm long, 2.5-5.7 cm broad, ovate or epileptic-oblong, acute or acuminate, often apiculate, glabrous, more or less pubescent especially when young, petioles 6-13mm long. The colour of leaves is green, odour is pleasant characteristic, and surface is smooth[4].

b) Microscopy

Leaf is composed of an outermost layer of thin walled, single layered epidermal cells covered by thin cuticle. Mesophyll differentiated into 2-3 layered palisade and 6-8 layered spongy parenchyma, the latter containing rosettes of calcium oxalate (druses). Epidermal peeling exhibits characteristics covering trichomes, multicellular (3 to 8 celled), uniseriate, bent and tapering at the end. Paracytic stomata are seen only on the abaxial surface. In the midrib region, collenchymas is present below the upper epidermis and above the lower epidermis. In the centre, xylem elements are arranged in an arc and
Phloem occurs on both sides of it. Many idioblasts with crystals are found in the ground tissue [4].

Figure 2
Microscopy of leaf of *Tylophora indica*. A. TS of Mid-rib portion, B. Section through Lamina region, C. Upper epidermal peel showing trichome, D. Lower epidermal peel showing multicellular trichomes.

Chemical constituents

The active constituents of *Tylophora indica* are phenanthroindolizidine alkaloids like tylophorine, tylophorinine, tylophorinidine and septidine. Recently some rare alkaloids namely tyloindicines A, B, C, D, E, F, G, H, I, and J, desmethyltylophorine, desmethyl tylophorinine, isotylocrebrine, anhydroustylophorinine, anhydrous-dehydrotylophorinine, γ-fagarine, skimmianine, 14-hydroxyisotylocrebrine, 4,6-desmethylisodroxy-o-Methyltylophorinindine have been reported. The non-alkaloidal compounds isolated from *Tylophora indica* are kaempferol, quercetin, α- and β- amyrins, tetratriacontanol, octacosanyl octacosanoate, sigmasterol, β-sitosterol, tyloindane, cetyl-alcohol, wax, resin, couchone, pigments, tannins, glucose, calcium salts, potassium chloride, quercetin and kaempferol. Steam distillation of an alcoholic extract of the air-dried root powder gave p-methoxysalicyaldehyde and a small amount of oily matter [11].
Figure 3
Chemical structures of chemical constituents found in Tylophora indica.

Phytochemical Studies

The drug Tylophora indica contains major chemical constituents Tylophorine, kaempferol, α-amyrin and quercetin, other major alkaloids like tylophoridine, desmethyltylophorine, desmethylylophorinine, desmethylylophoridine, dehydrotylophorine, anhydrousdehydrotylophorinine. Other alkaloids (+)-Septicine and (+)-isotylocrebrine from fresh leaf [4]. The new alkaloids include tyloindicines A-E, (+)-14-hydroxyisotylocrebrine and 4-6-desmethylysootylocrebrine. Tylophorine, 6-desmethyltylophorine, tylophoridine and 5-hydroxy-0-methyltylophoridine were the known alkaloids. Structural studies indicate that apart from tylophorindicne B, all alkaloids possess the dibenzo-{f,h} pyrrolo {1,2b} isoquinoline skeleton but differ in the number, nature and distribution of the oxygen bearing substituents, in the presence or absence of C-13a or benzylic hydroxyls and an angular methyl function. Tylophorine B possess a cleaved substituted phenanthrene moiety nucleus and an angular methyl group on the indolizidine portion [12]. Although tylophora alkaloids are structural analogs, their potency of cytotoxicity, selectivity against NF-kB signalling pathway, and their inhibitory effects against protein and nucleic acid synthesis are different. Because they don’t have an identical spectrum of targets, the studied are structural but may not be functional analogs [13].
Figure 4
Chemical structures of tylophora alkaloids and phenanthrene-based tylophorine derivatives.
Pharmacological studies

Hepatoprotective activity:

The methanolic extracts of Tylophora indica leaves was screened for hepatoprotective activity in carbon tetrachloride induced hepatotoxicity in albino rats. Tylophora indica leaves exhibited significant reduction in serum hepatic enzyme when compared to rats treated with carbon tetrachloride alone\[^{14}\]. The hepatoprotective activity of alcoholic (ALLT) and aqueous (AQLT) extracts of leaves of Tylophora indica against ethanol-induced hepatotoxicity. Ethanol induced significant changes in physical, biochemical, histological, and functional liver parameters. Pretreatment with ALL T and AQLT extract significantly prevented the physical, biochemical, histological and functional changes induced by ethanol in the liver\[^{15}\].

Lysosomal enzyme inhibiting activity:

The flavone fraction from Tylophora indica leaves showed significant dose dependent lysosomal enzyme inhibiting activity against adjuvant-induced arthritis at 20-50 mg/kg. Flavone fraction showed statistically significant inhibition of arthritis lesions (p<0.05) from day 18, (p<0.025) from day 20 and (p<0.001) from day 21 onwards in the adjuvant-induced arthritis studies which was compared to response of standard drug indomethacin\[^{16}\].
Antiallergic activity:

The anti-allergic effect of *Tylophora indica* was compound with that of disodium cromoglycate on perfused rat lung in sensitized rats by observing the changes in the volume of the perfusate per minute. Administration of aqueous extract of *Tylophora indica* and disodium chromoglycate during perfusion of sensitized rat lung significantly increased the rate of flow. The action of *Tylophora indica* may be due to direct bronchodilator property and membrane stabilising and immune-suppressive effects[17].

Diuretic activity:

Aqueous and alcoholic extracts of *Tylophora indica* leaves were tested for diuretic activity in rats. The aqueous and alcoholic extracts of *Tylophora indica* leaves possess good diuretic activity. It is investigated that ethanol is most effective in increasing urinary electrolyte concentration of all the ions i.e sodium, potassium and chloride followed by chloroform and aqueous extracts while other extracts did not show significant increase in urinary electrolyte concentration[18].

Immunomodulatory activity:

Studies with tylophora alkaloids had shown that they inhibit cellular immune response like contact sensitivity to dinitrofluorobenzene and delayed hypersensitivity to sheep red blood cells, in vivo. The alkaloids mixture suppressed IL-2 production at the lower concentrations. IL-1 production by activated macrophages on the contrary was doubled in the presence of inhibitory concentration dependent biphasic effect on con A induced mitogenesis. At lower concentrations they augment con A induced lymphoproliferation by enhancing IL-2 production. Inhibitory of proliferation at higher concentration of the alkaloids is due to inhibition of IL-2 production and activation of macrophages, which a cytostatic effect[19]. Crude extract of the leaves of *Tylophora indica* inhibited delayed hypersensitivity reaction to sheep red blood cells in rats when the alkaloid mixture was administered before and after immunization with these cells. The alkaloid mixture also inhibited contact sensitivity to dinitro-fluorobenzene in mice when given prior to or after contact sensitization. Lymphocytes taken from contact sensitized mice, when treated with tylophora alkaloid in vitro and transferred into naive syngeneic hosts, could suppress the transfer of delayed type hypersensitivity (DTH) response. However, the tylophora alkaloids could not suppress primary humoral (IgM)
immune response to SRBC in mice at the same dose. These studies suggest that tylophora alkaloids suppress cellular immune responses when administered at any stage during the immune response\[^{[20]}\].

**Mast cell stabilisation activity:**

The total alkaloids of *Tylophora indica* were tested for mast cell stabilising effect in comparison with disodium cromoglycate by challenging against three different mast cell degranulators, diazoxide, carbachol and polymixin B, invitro. The results suggest that tylophora alkaloids may have similar mechanism of action disodium cromoglycate through cyclic AMP\[^{[21]}\].

**Anti-Cancer Activity**

Tylophorine not only retards the S-phase progression but also dominantly arrests the cells at G1 phase in HepG2, HONE-1, and NUGC-3 carcinoma cells. Moreover, tylophorine treatment results in down regulated cyclin A2 expression and overexpressed cyclin A2 rescues the G1 arrest by tylophorine. Thus, we are the first to report that the downregulated cyclin A2 plays a vital role in G1 arrest by tylophorine in carcinoma cells\[^{[22]}\].

**Anti-Tumor Activity**

Tylophorine analogs had an inhibitory effect on cyclic AMP response elements, activator protein-1 sites, or nuclear factor kappaB binding site-mediated transcriptions. In summary, these tylophorine analogs are a unique class of antitumor compounds that have a mode of action different from known antitumor drugs\[^{[23]}\]. Polar phenanthrene-based tylophorine derivatives (PBTs) were designed, synthesized and evaluated as potential antitumor agents. The newly synthesized PBTs were evaluated for cytotoxic activity against the A549 human cancer cell line. Among them, N-(2,3- methylenedioxy-6-methoxy-phenanthr-9- ylmethyl)-1-2-piperidinemethanol and N-(2,3-methylenedioxy-6-methoxyphenanthr- 9-ylmethyl)-5-aminopentanol showed the highest potency with IC50 values of 0.16 and 0.27 M, respectively, which are comparable to those of currently used antitumor drugs derivatives\[^{[24]}\].

**Antifeedant and antimicrobial activity:**

Crude and pure extracts of *Tylophora indica* were investigated in view of antifeedant and antimicrobial activity. Pure compounds displayed strong antibacterial
activity at lower concentrations in all tested bacterial strains except E.coli. while all the crude and pure compounds showed antifungal activity against Aspergillus niger, Aspergillus fumigates and Trichoderma virdae, the pure compounds had strong antifungal activity compared to crude extracts[25].

Anti-Asthmatic

A brief exposure of human peripheral leukocytes from asthmatic children to tylophorine (an alkaloid occurring in Tylophora asthematica) caused the stimulation of adenyl cyclase. This effect was not observed in the leukocytes from the nonasthmatic children or adults[26].

Plant tissue culture

An efficient procedure has been developed for inducing somatic embryogenesis from mature leaves of Tylophora indica (Burm f.) Merill., and important medicinal plant. Leaf sections were initially cultured on Murashige and Skoog’s (MS) medium supplemented with thidiazuron (TDZ) in addition with 2,4-dichlorophenoxy acetic (2,4-D), particularly 0.5 μM TDZ, along with 1.5 μM 2,4 D was very effective in inducing somatic embryos. Plant were regenerated from in-vitro somatic embryos plated on semisolid medium devoid of growth regulators. Plantlets were obtained in 65% of the cultures with 2% sodium alginate coated embryos and control embryos showed 90% germination[27]. Organogenesis callus formulation from immature leaf pieces was obtained by using Murashige and Skoog (MS) medium supplemented with 7 M 2,4-dichlorophenoxyacetic acid and 1.5 M 6- benzyladenine. On the medium 92% explants produced callus. The optimal hormone combination for plantlet regeneration was 8 M thidiazaron, at which shoot buds were originated from 100% of the cultures, with an average of 66.7 shoots per culture. For roots formation half-strength MS-medium supplemented with 3 M indole-3-butyric acid was used. Plants were transferred to soil, where 92% survived after 3mol of acclimatization[28]. New and efficient transformation system for Tylophora indica using Agrobacterium rhizogenes strains LBA9402 and A4 to infect excised leaf and stem explants and intact shoots at different sites. The induction of callus and transformed roots was dependent on the bacterial strain, explant type and inoculation site used. Transformed roots were induced only in explants infected with A. rhizogenes strain A4, while an optimal transformation frequency of up to 60% was
obtained with intact shoots inoculated at the nodes. Root growth and the production of tylophorine, the major alkaloid of the plant, varied substantially among the nine root clones studied. Both parameters increased over time in liquid cultures, with maximum biomass and tylophorine accumulation occurring within 4-6 weeks of growth in fresh medium. Interestingly, in liquid culture, the culture medium also accumulated tylophorine up to concentrations of 9.78+/−0.21 mg l(−1) [29]. Protoplast culture and plant regeneration of an important medicinal plant Tylophora indica were achieved through callus regeneration. Protoplast were isolated from leaf mesophyll cells and cultured at a density of 5×10⁵ protoplast per gram fresh weight, which is required for the highest frequency of protoplast division (33.7%) and plating efficiency (9.3%). The calli developed shoot buds after 3-4 wk, and the frequencies of calli forming shoots varied from 5% to 44%. Optimum shoot regeneration occurred on MS medium supplemented with 5 M TDZ and 0.4 M NAA. On this medium 44% cultures responded with an average number of 12 shoots per callus. Whole plants were recovered following rooting of shoots in ½ MS medium supplemented with 3 M indole 3-butyric acid [30]. Tylophora indica plants have been shown to contain phenanthroindolizidine alkaloids of the tylophorine type. Cinnamic acid-[2-14C] was incorporated efficiently into these alkaloids supporting the hypothesis that ring A and C-10 and C-6 of tylophorine are derived from phenylalanine [31].

REFERENCES


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