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PHYTOCHEMICAL VARIATION IN *TYLOPHORA INDICA* (ASCLEPEADACEAE) LEAVES COLLECTED FROM DIFFERENT REGIONS

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The main objective of this study was to find out the best source of secondary metabolites especially tylophorin and stigmasterol. So the plants of *Tylophora indica* were collected from different regions(Jaipur,Ananad,Navsari,Bharatpur,Mumbai, Nagpur) in the month of March .Tylophorin and stigmasterol content were analyzed from the leaf part of all the plants collected from different regions. The quantification of both the compounds were carried out by using HPTLC analysis with their reference compound of tylophorin and stigmasterol and it was observed that tylophorin content was maximum in plant collected from Mumbai region, whereas in the case of stigmasterol it was highest in Bharatpur region's samples(tylophorin-088%; stigmasterol.0.0099%).This study is useful for the pharma industries.

Keywords : Stigmasterol; *Tylophora indica;* Tylophorin.

Introduction

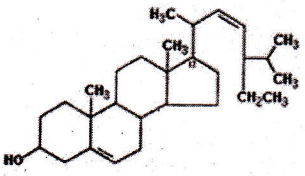
Genetic diversity plays an important role in plant conservation and their survival in adverse conditions. Many environmental factors such as precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow-cover, length of the vegetation period, and the intensity of radiation under clear sky conditions have been reported to differ between low and high altitude sites[1]. Moreover, study on phytochemicals of wild populations of plants at different altitudes were performed, and it is not conclusive whether the observed variations are a response of individual plants to environmental factors related to altitude or a genetic adaptation of the populations growing at different altitudes to their specific environment[2-4].

Plants may produce as much as 100,000 small molecules[5], which include primary metabolites that are present essentially in all plants resulting from primary metabolic activities, but the secondary metabolites that are specific to certain plant species, are produced in small quantities, have geographical impact on their production and are generally produced in a particular plant part[6].These compounds are the result of the secondary metabolic pathways that take place in certain plant species. For plants, little effects have been attributed to these potential substances like, defense against microorganisms, insects and herbivores. While some of them give plants their odors and pigments many of them are responsible for plant flavors but for humans, at instances, they become life saving drugs[7].The Chemotaxonomy and geographical distribution of tropane alkaloid has been studied in *Datura* sps.

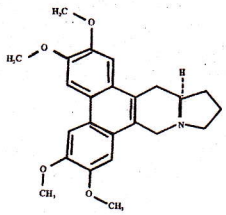
Tylophora indica (Burm. f.) Merill. (Asclepiadaceae) commonly known as "Antmool" is an important medicinal plant, traditionally used as a folk remedy in treatment of bronchial asthma, bronchitis, rheumatism, allergies and inflammation. The roots and leaves contain 0.2 to 0.46% therapeutically important alkaloids tylophorine,tylophorinine and tylophrinidine. Major alkaloid tylophorine has immunosuppressive, anti-inflammatory[8] anti-tumor[8] stimulant of adrenal cortex[9] and anti-amoebic[10] properties. Prior to this hepato protective activity of alcoholic and aqueous extracts of leaves of *T. indica* have been reported[11,12]. Tylophorine and its analogs are phenanthro indolizidine alkaloids, many of which have been isolated from plants of the family Asclepiadaceae, including members of the genus *Tylophora* that are native to India and Southeast Asia.

Phytochemical screening of the methanolic leaf extract of the plant revealed the presence of tannins, phytosterols (stigmasterol), saponins, flavonoids, carbohydrates and alkaloids (tylophorin)[13]. The main aim of the present work was to examine the content of stigmasterol and tylophorin from *Tylophora indica* plant that has been collected from six different region of India (Jaipur, Ananad, Navsari, Bharatpur, Mumbai, Nagpur)

and to find out the best source of both the natural products among them. Leaves were selected as the experimental material in the present study because this part of *Tylophora indica* has been used as medicine for asthama, inflammation, from ancient time. Stigmasterol is used as a precursor in the manufacture of semi synthetic progesterone[14-15], a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D_3[16]. The Upjohn company used stigmasterol as the starting raw material for the synthesis of cortisone[17,18]. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers. Studies have also indicated that a diet high in phyto sterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It was demonstrated that it inhibits several pro-inflammatory and matrix degradation mediators typically involved in osteoarthritis induced cartilage degradation[19]. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties[9].



$C_{24}H_{27}NO_4$ Tylophorin



$C_{29}H_{48}O$ Stigmasterol

Material and Methods

The plant materials were collected from various geographical region of India such as Anand, Nagpur, Bharatpur, Mumbai, Navsari and Jaipur in the month of March. Powdered (100g) leaves of *Tylophora indica* was defatted exhaustively separately with petroleum ether (60-80°C) . The residue was extracted with ethanol for stigmasterol estimation while in ethanol : methanol (8:2)for tylophorin (Fig.1). The Thin Layer Chromatographic analysis with the reference compounds of stigmasterol and tylophorin were carried out separately along with their resoective reference compound .TLC chamber was saturated with solvent system(Toluene: Diethylamine: Ethyl acetate; 14:2:2) for 15 min prior to use. 0.1mm thick silica del plates were used as stationary phase and Toluene: Di ethylamine: Ethyl acetate (14:2:2) was used as mobile phase. The confirmation of alkaloid was done by spraying the developed chromatogram with Dragendorf's reagent which gave the brick red colored five spot. The tylophorin was further confirmed by running TLC plate with standard tylophorin (Allexis Co. New Delhi).A very small amount of methanol was poured into the dried extract in the Petri plates and this was further used for TLC analysis. Further confirmation of presence of tylophorin was carried out by using Co-TLC with standard tylophorin (Rf value 0.59). The developed chromatograms were observed in UV light at 254nm (Fig. 2) ,which gave bright yellow color. The further confirmation of tylophorin was done by using HPTLC. In the case of analysis of stigmasterol ,TLC was used in the same way (solvent system -Hexane : acetone; 8:2). Developed plates were sprayed with 5% of sulphuric acid which gave a characteristic grey color (Rf-0.91) and suggested the presence of stigmasterol in the leaves of *T. indica.* The samples were subjected to the HPTLC analysis for quantitative estimation.

HPTLC analysis - HPTLC analysis was carried out in Anchrom Test Lab Pvt. Ltd using silica gel plates (60F254 Manufacturer E. MERCK KGaA),Sample application was carried out on CAMAG Linomat 5 Instrument (CAMAG Linomat 5 "Linomat5_080222" S/N 080222).Inert gas was used as spray gas. Sample solvent type was methanol. Dosage speed was 150nl/s and syringe size was 100µl and the analysis wave length was 430 nm. Toluene: Ethyl acetate: Diethyl amine (14:2:2) was used as mobile phase and Hexane :acetone (8:2) was used as mobile phase for stigmasterol analysis.

Results and Discussion

The quantitative analysis of both the compound were carried out with the help of HPTLC analysis with their respective standard compound separately. The quantitative estimation of both compounds were calculated by using their peak area. In literature,evaluation of ()-(S)-tylophorine [DCB-3500 (NSC-717335)] and its analog DCB-3503 (NSC-716802) for antitumor activity at the National Cancer Institute showed a fairly uniform and potent growth-inhibitory activity (GI518M) in 60 cell lines[20-27]. Genetic expression at the time of secondary metabolites production in plant cell governed by many factors and geographical distribution is one of them. Thus, a variation in tylophorin as well as stigmasterol content has been seen among all the samples used. It was observed that tylophorin content was maximum in plants collected from Mumbai region, whereas in the case of stigmasterol it was highest in Bharatpur region's samples (Table 1, Fig. 3). So it can be concluded that *T. indica* collected from

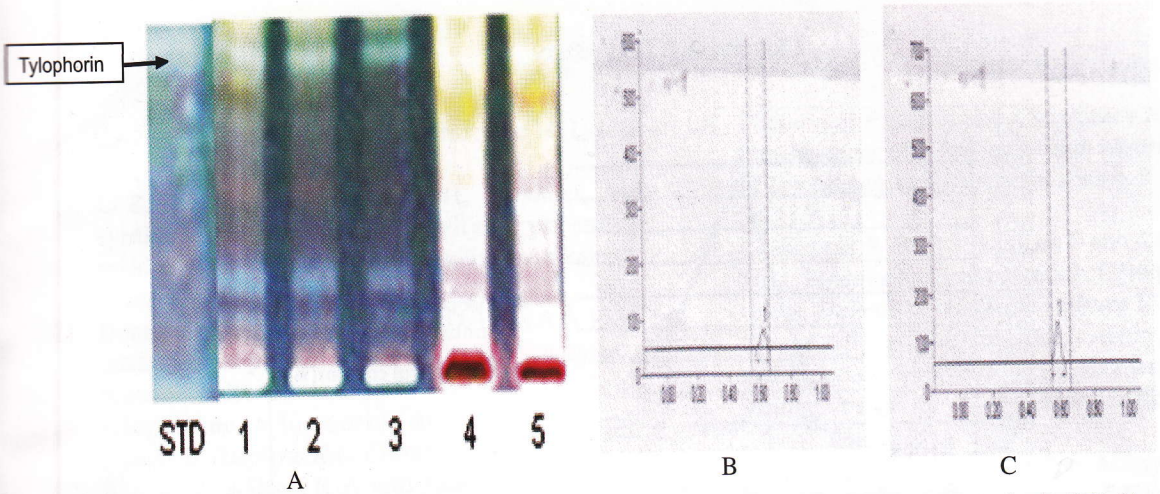


Fig.1. Showing the HPTLC analysis of tylophorin from methanol : ethanol (8;2) leaf extract and tylophorin STD (A-Finger printing, B-Chromatogram of leaf, C-Std tylophorin at 254nm.

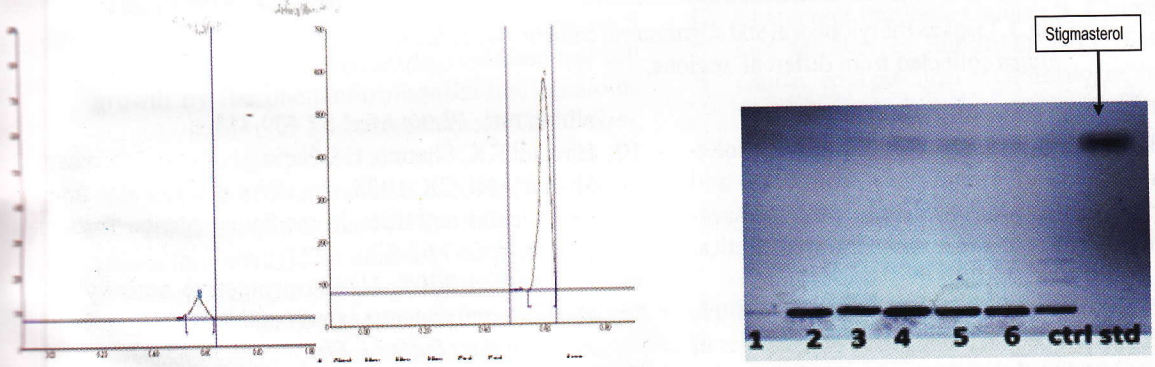


Fig.2. Showing the HPTLC analysis of ethanolic extract of *Tylophora indica* leaves of different area with std stigmasterol (ChromatogramA-Leaves ethanolic extract, B-STD),C- HPTLC Fingerprinting at 254nm.

Table 1. Depicts the tylophorin and stigmasterol content (%) of *Tylophora indica* leaves collected from different regions. Mean ± s.e.of three replecates.

Sample	Tylophorine content(%)	Stigmasterol content(%)
Anand	0.029±0.004	0.00162±0.031
Nagpur	0.013±0.023	0.00145±0.067
Bharatpur	0.021±0.016	0.0099±0.024
Mumbai	0.088±0.062	0.0089±0.019
Navsari	0.041±0.093	0.0065±0.063
Jaipur	0.052±0.017	0.0071±0.007

Mumbai region is good for tylophorin production while the same plant collected from Bharatpur region is well suited for the stigmasterol production. *T. indica* has been well examined biotechnologically as well as phytochemically[28-37]. Hence,it can be concluded that *T. indica* grown in these two regions are favorable for the production of their respective natural product synthesis. These results can be helpful for the pharma industries as also for the cryopreservation of the elite germplasm.

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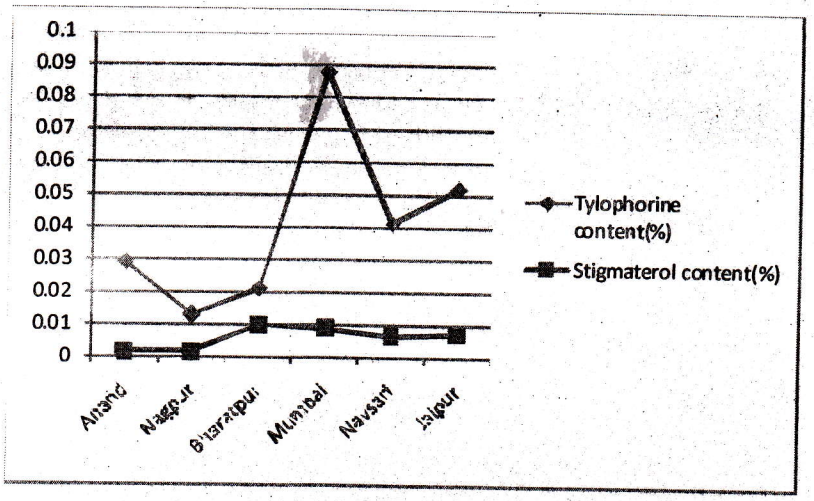

Fig.3. Depicts the tylophorin and stigmasterol content (%) of leaves of *Tylophora indica* collected from different regions.

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