ISSN 0974 – 5211

Journal of Natural Products

Volume 8 (2015)

www.JournalofNaturalProducts.Com

Research Paper

Phytochemical studies of *Tylophora* species of Goa

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(Received 27 April 2015; Revised 17 May – 25 Oct. 2015; Accepted 07 Nov. 2015; On-lined 29 Nov. 2015)

ABSTRACT

Species of *Tylophora* (Family-*Asclepiadaceae*) are documented for their medicinal uses right from folklore to modern day medicine. Many researchers have contributed in the characterization of phytochemical substances present in the plants belonging to this family. Some reports claim that closely related species are useful for treating the same ailment. Hence it was felt to test the species of *Tylophora* through simple morphological, pharmacological, spectrophotometrical and chromatographical tests. Accordingly when morphological and phytochemical studies were carried out on the species found in Goa it showed results which can be used for the standardization, quality control and preliminary way of authenticating the closely related species. Some prominent morphological variations were seen within *T. indica* species with respect to leaf morphology. It showed absence of latex and low amount of alkaloids when compared to *T. dalzelli*. Chromatographic studies showed presence of two alkaloids in *T. indica* one being Tylophorine. *T. dalzelli* also showed two alkaloid spots, second being not very clear. Similar medicinal properties could be attributed to phytochemical similarities with respect to one spot present in both the species. This finding will be helpful to select the alternate or closely related species with similar properties and scientifically validate the use of plant in the treatment of diseases.

Key words: *Tylophora indica*; Phytochemical; Goa.

INTRODUCTION

'*Tylophora*' is a genus of slender climbing perennial plants commonly known as "Panaceae Twine". Some species found in India are *Tylophora indica, T. rotundifolia, T. fasciculata, T. apiculata, T govanii, T. anomala, T. helferi, T. macrantha, T. iphisia, T. cappardifolia, T. globifera, T. sylvatica and T. hetero-phyla* (Kaur and Singh, 2012; Rani et al., 2012). In Goa, only three species of *Tylophora* were recorded namely: *T. dalzelli, T. indica and T. fasciculate* (Rao, 1985). Goa is frequented by *T. indica* at specific shady and mesophytic locations whereas another closely related species *T. dalzelli* is commonly found as roadside weed. Though *T. indica* is most known for asthma treatment traditionally, it is also reported in the literature that *T. dalzellii* has the same medicinal properties. However, its chemical constituents are not known (Chopra et al., 1956). Similarly *T. dalzelli* is reported as a local herb for the treatment of asthma in Goa (Bhonsle, 1973). This is also used as a

folk remedy for dysentery and asthma and as an emetic, expectorant and diaphoretic in those regions. The efficient properties impressed the British medical practitioners like Dr. Russel, Dr. Kirck Patrick etc. and they administered it to their patients during their regular practices (Shah and Kapoor, 1974).

It was also confirmed that alkaloid "Tylophorine" which was isolated from the leaves has significant effect on patients suffering from bronchial asthma (Shivpuri et al., 1968). Significant activity of the extracts of stem and leaf were observed against two standard transplantable tumors, lymphoid leukemia L1210 and lymhocytic leukemia P388 by Chitnis et al., (1972). Looking at the exemplary medicinal uses of *T. indica* species, there is already a concern about it's over exploitation and decline in the wild population (Jayanthi and Mandal, 2001).

WHO, 2011 estimated that 235 million people suffer from asthma and this is common chronic disease among children. Synthetic drugs like steroids used to treat asthma have shown many side effects during long-term usage. This has made the practitioners to depend on herbal drugs to control not only asthma but also other related diseases (Rani et al., 2012). It becomes necessary then to provide the best or alternate source of herbal drugs as an important natural source for the treatment of respiratory diseases.

Though Najafi and Deokule (2010) carried out pharmacognostic studies on *T. dalzelli* there is no literature on comparative analysis neither of secondary metabolites nor of their activity with regards to asthma and other diseases.

Here for the first time we carried out morphological studies followed by preliminary phytochemical tests for comparison, identification and authentication of various species of *Tylophora* distributed in different regions of Goa.

MATERIALS AND METHODS

Plant collection: The species of genus *Tylophora* were collected from different districts of Goa North and South and were grown under ex-situ conditions. The plants were brought to the laboratory and identified with the help of The Flora of the Presidency of Bombay and Flora of Goa, Diu, Daman, Dadra and Nagarhaveli (Cooke, 1967; Rao, 1985). They were also confirmed at Botany department of Goa University.

Morphological studies: Comparative morphology method was followed for the morphological studies of the collected and identified plant species.

Extraction Procedure: For phytochemical investigation fresh leaves of collected species were washed thoroughly to remove all debris and soil particles associated with. These leaves were then oven dried and powdered. Organic-solvent extraction protocol was followed (Cseke et al., 2007). 10g of leaf powder was placed in Soxhlet's apparatus along with 200ml 90% Methanol and 2% Tartaric acid solution and incubated at 60°C till it became colorless. Further, the extract was evaporated in an evaporating dish on a water bath at 50-60°C to obtain a concentrate.

Phytochemical Studies: The above extract was then subjected to various phytochemical analyses to test the presence of phytoconstituents like tannins, saponins, alkaloids, carbohydrates, phenolics, flavanoids and terpenoids (Rawat and Upadhyaya, 2013; Tonk et al., 2011; Harborne, 1998; Dutta, 1999).

Test for alkaloids: 10mg of solvent free extract was stirred with few ml of dilute HCL and filtered. The filtrate was tested carefully with reagents as follows:

Mayer's reagent test: Took few ml of filtrate, a drop or two of Mayer's reagent was added, by the side of the tube.

Dragendorff's reagent test: To a few ml of filtrate, 10ml of Dragendorff's reagent was added.
Test for Carbohydrates: The extract was dissolved in 2.5ml of water and filtered. The filtrate was subjected to the following tests:
Benedict's test: To 0.5ml of filtrate 0.5ml of Benedict's reagent is added and boiled for 2 mins.
Molish's reagent: To 2ml of filtrate, 2 drops of alcoholic solution of α-naphtol are added and shaken well and 1ml of H_2SO_4 was added slowly along the sides of the test tube.
Test for Saponin: 25g extract is taken in 10ml distilled water and shaken vigorously for 15 min.
Test for phenolics and tannins:
Ferric chloride test: 50mg of extract is dissolved in 2ml distilled water and a few drops of 0.1% Ferric chloride were added.
Lead acetate test: 50mg of extract is dissolved in distilled water and 3% of Lead acetate solution is added.
Test for flavonoids: 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4.
Test for terpenoids: 5ml of extract was mixed in 2ml of Chloroform, and conc. H_2SO_4 was added to form a layer.
Tylophorine analysis: The absorption spectra of collected plant extracts were measured at the wavelength of 590 using the visible spectrophotometer. Organic solvent methanol was employed as a solvent for its spectrophotometric analysis (Harborne, 1998; Dutta, 1999). The values so obtained were used to calculate the amount of Tylophorine, using the formula given below (Tonk et al., 2011):

$$c = E\ 590 \times (\varepsilon \times e)^{-1} \times 100$$

- c =Tylophorine content; e =Amount of sample; E590 =Spectro value at 590 nm; ε = Extraction coefficient (718)

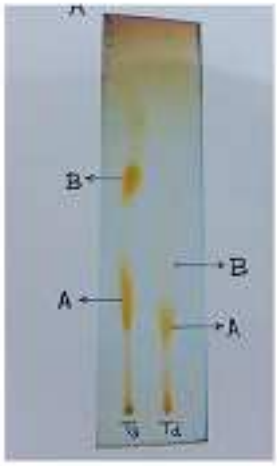
TLC: On the basis of spectrophotometric and phytochemical analysis, qualitative and quantitatively viable species were selected for chromatographic studies. TLC was carried out using solvent system Chloroform: acetone: diethyl-amine in the ratio 5:4:1. The spots were compared and the R_F values were calculated (Balasubramanian et al., 2010; Cseke et al., 2009; Harborne, 1998; Stahl, 1965).

RESULTS

Morphological studies: Collected plants from different regions of Goa were identified as two species of Tylophora one *T. indica* and other *T. dalzelli*. Morphological studies showed small variations between two species as well as within *T. indica* collected from various regions. Out of four species collected of *Tylophora*, three were identified as *T. indica* obtained from Pernem, Valpoi and Rivona whereas one of them as *T. dalzelli* from Khotigao. The difference between the two species *T. indica* and *T. dalzellii* was with respect to small flowers and presence of latex. Some prominent morphological variations were also seen within *T. indica* species obtained from Valpoi, which showed considerable differences in leaf morphology. The leaf apex in *T. indica* was mucronate and leaf base was much cordate and smaller **(Figure 1).**

Fig.1 Flowering A- *Tylophora indica* (Rivona) ; B- *Tylophora indica* (Valpoi) showing mucronate apex and cordate base ; C- *T. dalzelli* (Khotigao).

Fig. 2 TLC plate Tv-T. indica (Rivona); Td- T. dalzellii (Khanigao)



Phytochemical tests: Plant extract obtained after Soxhlet's extraction process subjected to phytochemical tests showed the presence of Alkaloids, Carbohydrates, Saponins, phenolics, tannins, flavonoids and terpenoids in all the species **(Table 1).**

Table-1: Phytochemical tests of *Tylophora* species extract.

Test	Observation	Inference			
		T_P	T_V	T_R	T_K
Test for alkaloids					
Mayer's test	A white or creamy precipitate appeared	+	+	+	+
Dragendorff's test	A prominent yellow precipitate was formed	+	+	+	+
Test for Carbohydrates					
Benedict's test	A brick red colored precipitate appeared	+	+	+	+
Molish's test	A violet ring was formed	+	+	+	+
Test for saponins	A 2cm layer of foam is formed	+	+	+	+
Test for phenolics and tannins					
Ferric chloride test	A dark green colour appeared	+	+	+	+
Lead acetate test	Formation of bulky white precipitate	+	+	+	+
Test for flavonoids	Yellow coloration found which disappeared on standing	+	+	+	+
Test for terpenoids	Reddish brown coloration of the inter face was formed	+	+	+	+

- (+) showing presence and (-) showing absence of the phyto constituent.
- T_P-*T. indica* (Pernem); T_v-*T. indica* (Valpoi); T_R-*T. indica* (Rivona); T_k - *T. dalzellii* (Khotigao)

Spectrophotometric analysis: The amount of alkaloid is highest in *T. dalzelli*. Within *T. indica* species, the one collected from Rivona showed high amount of alkaloids **(Table 2).**

Table -2: Alkaloid contents (mg/ml) in *Tylophora* sps.

Sample	Value of alkaloid in mg/ml
T. indica (Pernem)	0.355
T. indica (Valpoi)	0.344
T. indica (Rivona)	0.356
T. dalzellii (Khotigao)	0.711

Thin layer chromatography: On conducting TLC, it was observed that the solvent system which consisted of Chloroform: Acetone: Diethyl-amine in the ratio 5:4:1

gave prominent results. Two spots of Rf values were obtained on TLC plate after treating with Dragendorff's reagent. 0.35 (A) and 0.7 (B) was detected in *Tylophora indica* from Rivona whereas *T. dalzelli* showed spots at 0.3 (A). The B spot highlighted may be present in a very small amount or may not exist (**Figure 2**).

DISCUSSION

T. indica (Burm.f.) Merr. or Indian Ipecac is commonly known as Antamul in Marathi and Pitvel in Konkani (local language). *Tylophora dalzellii* is known as Dalzell Ipecac, and commonly known as Lhan Pitmari in Konkani. *Tylophora indica* was limited to certain pockets of Areca nut plantations having sandy soil, low temperature and humidity. *T. dalzelli* in contrast was found to be growing as weed climber along the pathways, in the forest and open areas as well. There were also variations within *T. indica* sp. collected from Pernem, Rivona and Valpoi. Species from Pernem and Rivona showed many similarities with regard to the leaves and flowers whereas the difference was much notable when compared it with the species from Valpoi. The variations within species suggest greater persistence in a given geographical area (Sebastian et al., 2010).

Further Phytochemical studies carried out showed presence of alkaloids, carbohydrates, saponins, phenolics, tannins, flavonoids and terpenoids in leaf extracts of all the selected species contrary to the work carried out by Najafi and Deokule, 2010 on *T. dalzelli* wherein absence of flavonoids and glycosides are reported in the leaves of *T. dalzelli.* Presence of flavonoids in collected species for our studies can be attributed to geographical locations from where plants were collected and also phase of plant growth. It is mentioned that there could be certain instances wherein certain component would be absent in the plant species and the part of the plant under investigation like absence of carbohydrates from seeds of *M. ferrea* (Rawat and Upadhyaya, 2013).

Spectrophotometrical analysis further gives the idea of the amount of total alkaloid present in the leaf extract. It is found in the concentration of 0.355, 0.344, 0.356 and 0.711 in *T. indica* from Pernem, Valpoi, Rivona and Khotigao respectively. *T. dalzelli* gave almost double the amount of alkaloid. This result further differentiates between the two species. Similar analysis was performed which analyzed the Hypericin content in the clones of *Hypericum perforatum* (Tonk et al., 2011).

On the basis of qualitative estimation within the species of *T. indica* the species collected from Rivona and from Khotigao were selected for chromatographic estimation. TLC performed revealed further understanding of its components. The separation carried out using solvent system Chloroform: acetone: diethyl-amine in the ratio 5:4:1 gave clear differences with respect to alkaloids present in the two species. *T. indica* showed two alkaloids one of them confirmed to Tylophorine (Sharma and Anand, 2011) whereas *T. dalzelli* showed only one prominent alkaloidal spot. No references are available about TLC profile of *T. dalzelli* so far.

CONCLUSION

Studies carried out on collected Tylophora species shows variations within *T. indica* species and between *T. indica* and *T. dalzelli* with respect to their morphological and chromatographic properties. Present work can serve as basic methods for identifying and authenticating *T. indica*. These findings are useful in standardization, quality control and preliminary way of authenticating the closely related species of highly useful medicinal plants thus avoiding or detecting adulteration of medicinal product. This work further envisages the need to study *T. dalzelli* for its antiasthamatic

properties as done on *T. indica*.

Acknowledgement: The authors are grateful and acknowledge the facilities and infrastructure provided by the Department of Botany, Parvatibai Chowgule College to carry out this research.

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