Comparative Study of *Tragia involucrata* L. and *Tragia* Spp. by Using Preliminary Standardization Techniques

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***Abstract*—** *Since ancient times humans have been using plants as medicine in different formulations to treat various ailments. Tragiainvolucrata L. [TI] commonly known as Welkahambiliya (Sinhala) andIndian stinging nettle (English) is a widely used indigenous medicinal plant. Experimentally, it shows analgesic, wound healing, anti-inflammatory, anti-microbial, psychopharmacological, anti-cancer, anti-diabetic, hypolipidaemic, diuretic and antioxidant activities. In industry another Tragia spp. is used as substitute for TI. Morphologically, both plants consist of stinging hair which irritates skin. Difference between the two plants is stem type. TI is a vine and Tragia spp. starts to grow as a shrub and twines around a support after it grows to about 1 meter in height. Present study aimed to investigate physic-chemical and preliminary phytochemical screening of powdered whole plant of both Tragia species as aninitial study to compare the plants and investigate whether Tragia spp. can be used as substitute for TI. Hot and cold extractions of both species were prepared using solvents with different polarities dichloromethane, ethyl acetate, ethanol, methanol and water. Physico-chemical parameters such as moisture content, total ash content, acid soluble and water insoluble ash content were determined. Both plant materials examined showed many physico-chemical and phytochemical similarities. Extractive values were determined in hot and cold extractions of each solvent. In phytochemical screening presence of alkaloid, coumarins, flavonoids, glycosides, cardiac glycosides, steroid glycosides saponins, tannins and terpenoids were investigated. TLC was performed to hot and cold dichloromethane, ethyl acetate, methanol, and water extracts of both species using different solvent systems. They were visualized under UV 254 nm 366 nm. It was observed that most Rf values were similar in both plant materials. These preliminary studies provide referential information regarding plant identification and also reveal that both plant materials have similarities in phytochemical compounds and TLC fingerprinting. Further in vivo biochemical studies should be performed to investigate efficacy of the two plant materials for their pharmacological activities.*

***Keywords*—** *Tragia involucrata, Tragia* spp., *Standardization*

I. INTRODUCTION

From primeval times medicinal plants have been used as medical remedies throughout the world. Even with the great advances in modern medicine still about 25% of all modern medicines are directly or indirectly derived from higher plants. Thus, it is evident that plants still play a major role in health care.

Tragia involucrata L. (TI) is commonly known as *Wel kahambiliya* (Sinhala) &Indian stinging nettle (English). Although it is not so popular among lay people as a medicinal plant it is widely used in indigenous medicine by Ayurvedic and indigenous medical practitioners in Sri Lanka. The plant is being greatly destroyed since it is considered as a weed and also because it irritates the skin due to stinging hair. Experimentally the plant shows a wide range of biological activities such as, anti-diabetic, anti-cancer, hypolipidaemic, diuretic, antioxidant, anti-inflammatory, analgesic, wound healing, psychopharmacological, nematicidal, larvicidal, anti-bacterial etc. In Sri Lanka two types of plants are used by the name *Wel kahambiliya.* TI is a vine and the other species recognized as *Tragia* spp. starts to grow as a shrub and twines around a support after it grows to about 1 meter in height. Both plants are used by traditional and Ayurvedic practitioners for similar medicinal actions. In Ayurvedic drug industry mainly the shrub variety is used.

The present study intends to investigate the physico-chemical and preliminary phytochemical screening of powdered whole plant of both *Tragia* species as an initial study to compare the two plants and to investigate whether *Tragia* spp. can be used as a substitute for TI.

II. METHODOLOGY AND EXPERIMENTAL DESIGN

Hot and cold extractions of both plant species were prepared using solvents with different polarities dichloromethane, ethyl acetate, ethanol, methanol, and water. Physico-chemical parameters such as moisture content, total ash content, acid insoluble and water soluble ash content were determined.

Extractive values were determined in hot and cold extractions of each solvent. During phytochemical screening presence of alkaloid, coumarins, flavonoids, glycosides, cardiac glycosides, steroid glycosides saponins, tannins and terpenoids were investigated. TLC were performed to hot and cold dichloromethane, ethyl acetate, methanol, and water extracts of both species using different solvent systems. They were visualized under UV 254 nm 366 nm.

- Test for Alkaloids - To 2 ml of extract, 1 ml of 1% HCl was mixed and shaken. Mayer's reagent was added drop by drop by the side of the test tube. White or creamy precipitate indicates presence of alkaloids.

- Test for Coumarins -About 0.5 ml of 1% KOH in absolute ethanol was poured into 1 ml of diluted extract. Extract turns yellow indicating presence of coumarin.

- Test for Flavonoids -Two tests were performed.
  - To 1 ml of extract about 0.5 ml of 10% Lead acetate was poured. Formation of a yellow precipitate indicates the presence of Flavonoids.
  - Dil. NH_3 was added to a portion of extract followed by addition of 1 ml of Conc. Sulphuric acid. Yellow colour indicates the presence of Flavonoid.

- Test for Glycosides - A volume of 2 ml of $CHCl_3$ was added to the extract followed by 2 ml of Acetic anhydride and Conc. H_2S0_4. Conc. H_2S0_4 should be added carefully. Reddish brown ring at the interface indicates the presence of Glycosides.

- Test for Cardiac glycosides (Keller-Kiliani test) - To 2 ml extract 1 ml glacial acetic acid, six drops of 10% ferric chloride solution and six drops of Conc. H_2S0_4 were added. Green-blue color indicates the presence of cardiac glycosides.
- Test for Steroid glycosides (Liebermann's test) -Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2S0_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

- Test for Saponins -To 0.5 ml extract 5 ml distilled water was added in a test tube and vigorously shaken. Persistent froth volume produced, checked each 10 minutes for 30 minutes, indicates presence of saponins.

- Test for Tannins -To 2 ml of extract, few drops of Ferric chloride were added. Blue-black precipitate indicates the presence of tannins.

- Test for Terpenoids -To 2 ml of extract, 2 ml of chloroform was added. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

III. RESULTS

Total ash content of TI was 8.15 % + 0.03 and *Tragia* spp. was 10.95 % + 0.03 and water soluble ash content TI was 1.90 % + 0.06 and Tragia spp. was 3.74 % + 0.05. Acid insoluble ash content was 0.80 % + 0.01 in TI and 0.67 % + 0.04 in *Tragia* spp.

Table 1. Values related to standardization parameters of

Parameter	*Tragia involucrata* L.	*Tragia* spp.
Moisture content	7.59 % $\pm$ 0.02	7.77 % $\pm$ 0.17
Total solids	92.41 %	92.23 %

Tragia involucrata L. and *Tragia* spp

Extractive values were high in hot water in both plant species which ranged from 28.2 % - 30.4 %. Methanol extractive value ranged from 6.2 % - 13.2 % in both plant species. Extractive values of ethanol ranged from 3.6 % - 9.4 %. Both Ethylacetate and Dicholomethane extractive values of both plants species ranged from 1.4 % - 2.7 %.

Chromatographic fingerprinting of *Tragia involucrata* L. and *Tragia* spp. In two different solvent systems are shown below.

- Ethylacetate 5: Cyclohexane 7.5: Dichloromethane 37.5

Figure 1. TLC fingerprint profiles of *Tragia involucrata* L. (left) and *Tragia* spp. (right) at 254 nm

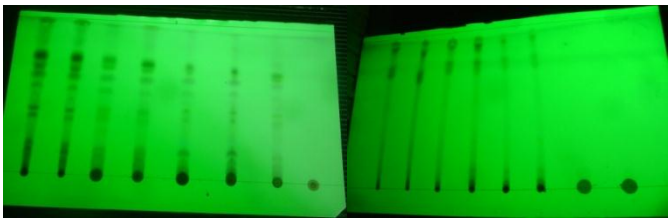
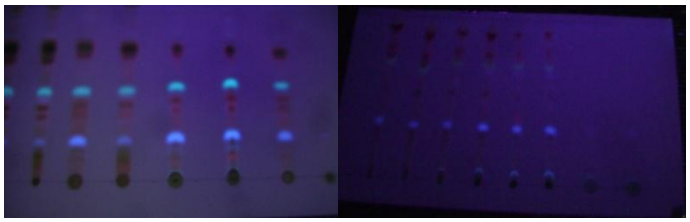
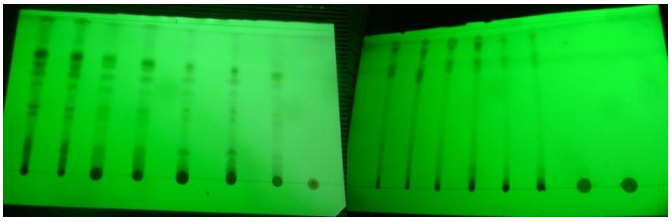


Figure 2. TLC fingerprint profiles of *Tragia involucrata* L. (left) and *Tragia* spp. (right) at 366 nm



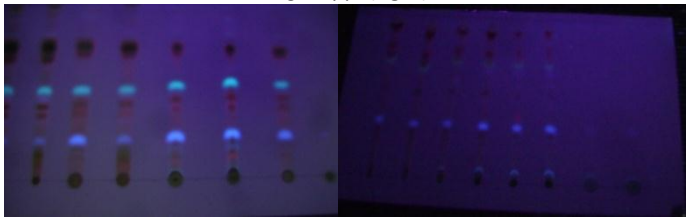
- Ethylacetate 15: Cyclohexane 7.5: Dichloromethane 27.5

Figure 3. TLC fingerprint profiles of *Tragia involucrata* L. (left)



and *Tragia* spp. (right) at 254 nm

Figure 4. TLC fingerprint profiles of *Tragia involucrata* L. (left) and *Tragia* spp. (right) at 366 nm



In phytochemical screening alkaloids were not detected in any of the extracts in both plant species. Coumarins were present in almost all extracts of both plant species. Flavonoids were present in water, methanol, and ethanol hot and cold extracts in both plant species and were absent in ethylacetate and dicholoromethane hot and cold extracts. Glycosides was present in almost all the extracts in both species. Cardiac glycosides were absent in water and methanol extracts and present in ethanol, ethylacetate, and dicholoromethane extracts in both species. Steroid glycosides were absent only in hot and cold water extracts and present in all other extracts. Saponins were detected in only water and methanol extracts in both species. Both Tannins and Terpenoids were present in all extracts of both plant materials.

IV. DISCUSSION AND CONCLUSION

Results of the physic-chemical parameters have been presented in Table 1. Extractive values of different solvent systems have been shown in Table 2. Results of the preliminary phytochemical screening studies have been presented in Table 3. TLC fingerprint profiles of TI and Ts are shown in figure 1 and 2 respectively.

The extractive value is useful for the evaluation of crude plant material as it gives an idea about the nature of chemical constituents present in the plant material. It is also useful for the estimation of chemical constituents soluble in a specific solvent used for extraction. In the present study, the extractive matters of both plants are significantly higher in the hot water extract compared to cold water extract. It is also evident that extractive values have considerably gone down in solvents with low polarity. Therefore, more chemical constituents appear to have dissolved in hot polar solvents compared to non-polar solvents.

The ash consists mainly of oxides of metals, salts and inorganic constituents. The ash value is useful in estimating the purity of a crude plant material. Acid insoluble ash indicates the presence of silica which shows the plant material being contaminated with earth or sand. Water soluble ash gives an idea about the water soluble salts present in the plant material. It is evident in this study that the total ash, water soluble ash and acid insoluble ash are comparatively higher in *Tragia* spp. than *Tragia involucrata* L.

Bio activity of a medicinal plant relies on the presence of phytochemicals or bio active compounds in medicinal plants. Table 3 depicts the presence of different bioactive compounds in both plant species in different extracts. Alkaloids were not detected in any type of solvent extractions. Tannins and terpenoids were present in all the extractions. Glycosides were not present in water extraction of *Tragia* spp. while it was present in TI water extraction. All the other phytochemicals were absent or present similarly in both plant species.

In conclusion the present study revealed similarities and differences between *Tragia involucrata* L. and *Tragia* spp. in terms of physico-chemical and phytochemical

parameters. These preliminary studies provide referential information in regard to the plants identification and it also revealed that both plant materials have similarities in phytochemical compounds and TLC fingerprinting. Further in vivo biochemical studies should be performed to investigate the efficacy of the two plant materials for its pharmacological activities.

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