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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING FOR SECONDARY METABOLITES IN TWO MEDICINALLY IMPORTANT PLANTS TINOSPORA CORDIFOLIA AND AERVA LANATA

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ABSTRACT

Tinospora cordifolia (Willd) Miers ex Hook. F. & Thoms and *Aerva lanata* are medicinally important plants due to the presence of numerous secondary metabolites. The notable medicinal properties reported are antidiabetic, antiperiodic, antispasmodic, antimicrobial, antiinflammatory, anticancer, antiarthritic, antioxidant, hepatoprotective, immunomodulatory activities. Preliminary phytochemical screening and secondary metabolite analysis was reported in the present study. The qualitative analysis showed the presence of steroids, alkaloids, flavonoids, phenolics, saponins, tannins etc. Quantitative analysis reveals higher amount of phenols (172.33±2.52), tannins (186.67±5.21), alkaloids (520.33±9.61) and flavonoids (169.33±8.33) in *T. cordifolia* when compared to *A. lanata*.

INTRODUCTION

Plants have been used in traditional medicine for thousands of years. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, homeopathy, Unani and Siddha. In large number of countries human population depends on medicinal plants for treating various diseases as well as for the source of livelihood. The main advantage of using medicinal plants is that, these do not cause any side effects when compared with synthetic drugs, because medicinal plants have high content of antioxidant compounds. This gives protective effects against diseases without reducing their therapeutic efficacy (Ragavendran *et al*., 2011). The Indian traditional system of medicine, especially Ayurveda has put forward a number of therapeutic claims on plant drugs. However it is important to conduct thorough investigation of many traditionally used medicinal plants with reference to modern system of medicine (Venkatesh *et al*, 2009). The World Health Organization estimated that 80% of the population in developing countries relies on traditional medicines, mostly plant drugs for their primary health care needs (Gaja Lakshmi *et al*., 2012; Appia Krishnan *et al*., 2009; Venkatesh *et al*, 2009). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body.

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The most important bioactive constituents present in the plants are alkaloids, tannins, flavonoids, saponins, steroids, terpenoids and phenolic compounds (Edoga *et al*., 2005; Mann, 1978). A large number of these phytochemicals shown to have inhibitory effects on most of microorganisms *in vitro* (Cowan, 1999). *Tinospora cordifolia (*Willd.) Miers is known as Amrita (Guduchi) belonging to the family *Menispermaceae* which is distributed throughout tropical Indian subcontinents and widely used in the Ayurvedic system of medicine "Rasayanas" to enhance the immune system and resistance against infections (Warrier *et al*., 2007). The notable medicinal properties are antidiabetic, antiperiodic, antispasmodic, antimicrobial, antiinflammatory, antiarthritic, antioxidant, skin diseases, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory, anaemia, urinary disorder and antineoplastic activities (Krishna *et al*., 2009; Panchabhai *et al*, 2008; Upadyay *et al*., 2010). *Aerva lanata* Linn. (Amaranthaceae family) known as polpala and it is a ethnomedicinally important plant exhibits anthelmintic, demulcent, antiinflammatory, antidiabetic, diuretic, expectorant, hepatoprotective and nephroprotective activities (Manokaran, 2008; Anantha *et al*., 2010). The present study aims at both qualitative and quantitative analysis of secondary metabolites in *Tinospora cordifolia* and *Aerva lanata*, as these plants are rich sources secondary metabolites.

MATERIALS AND METHODS

Collection of plant materials: Fresh leaf material of *Tinospora cordifolia* and *Aerva lanata* were collected during the month

of January from in and around Andhra university campus Visakhapatnam, Andhra Pradesh, India. Taxonomic identification of the plants was carried out with the herbarium present in the Department of Botany, Andhra University, Visakhapatnam.

Extraction of plant material: The fresh leaf materials of *Tinospora cordifolia* and *Aerva lanata* were collected respectively and washed thoroughly with running tap water and air dried under shade. After complete shade drying the plant material was grinded, the powder was kept in small plastic bags with paper labeling. The grinded plant material of about 5gm was crushed in 25 ml of sterile water, boiled at 50-60°C for 30 minutes in water bath and it was filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 5°C for further use. The filtrate was used for the phytochemical screening. The extraction was done by using soxhlation extraction method with analytical grade methanol as refluxing solvent. At the completion of extraction process, the plant extract was recovered from the mixture by distillation and stored at 4°C until further use. The percentage of plant extract content was calculated by using standard formula. Methanolic extract was used for the quantitative estimation of phenols, flavonoids, tannins and alkaloids.

Phytochemical Screening: Preliminary qualitative phytochemical screening was carried out with the following methods.

Steriods: One ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids (Gibbs, 1974).

Terpenoids: Two ml of extract was added to 2 ml of acetic anhydride and concentrated H_2SO_4. Formation of blue, green rings indicates the presence of terpenoids (Ayoola, 2008).

Fatty Acids: 0.5 ml of extract was mixed with 5 ml of ether. This extract was allowed for evaporation on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids (Ayoola, 2008).

Tannins: Two ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins (Treare and Evans, 1985).

Saponins: Five ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins (Kumar *et al*., 2009).

Anthocyanins: Two ml of aqueous extract is added to 2 ml of 2N HCl and ammonia. The appearance of blue-violet indicates the presence of anthocyanins (Paris and Moyse, 1969).

Leucoanthocyanins: Five ml of aqueous extract was added to 5 ml of isoamyl alcohol. Upper layer appears red in colour it indicates for presence of Leucoanthocyanins (Paris and Moyse, 1969).

Coumarins: Three ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins (Rizk, 1982).

Emodins: Two ml of NH_4OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins (Rizk, 1982).

Alkaloids: Aqueous extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Phenols: Aqueous extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols.

Flavonoids: Aqueous extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Quantitative phytochemical analysis

Total phenolic content

The total phenolic content was determined spectrophotometrically by the method described by Sadasivam and Manickam (1996). To 2ml of phenol extract 1.0 ml of Folin Ceocalteau reagent was added. After 3 minutes, 13 ml of distilled water was added. Later 2 ml of sodium carbonate (7.5%) solution was added and the volume was adjusted to 20 ml. The above mixture was kept for one hour for colour development and absorbance was recorded at 630 nm. The concentration of total phenolic content in plant extracts was calculated from the calibration curve of Gallic acid and it was expressed as Gallic acid equivalents/gram fresh weight. Each experiment has three replicates and the experiment was repeated thrice.

Total flavonoid content

Total flavonoid content was measured by aluminum chloride colorimetric assay described by Marinova *et al* (2005). One ml of plant extract was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10% $AlCl_3$ was added. At 6^{th} min, 2 ml of 1 M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The total flavonoid content in plant extracts was calculated from the calibration curve of catechin and it was expressed as Gallic acid equivalents/gram fresh weight. Each experiment has three replicates and the experiment was repeated thrice.

Tannin content

The tannin content was determined by the method given Sadasivam and Manickam (1996). To 1ml of plant extract 3.5ml of distilled water and 0.5ml of Folin-Denis reagent was added. Contents were allowed to mix then 1ml of saturated sodium carbonate solution was added. The final volume was made up to 10ml with distilled water. The solution was mixed well at room temperature and the absorbance was measured

against prepared reagent blank at 760 nm. Tannin content in plant extracts was calculated from the calibration curve of tannic acid (10-100 µg) and it was expressed as tannic acid equivalents/gram weight. Each experiment has three replicates and the experiment was repeated thrice.

Alkaloid content

Total alkaloid content in the extract was estimated by spectrophotometric method based on using Dragendorff's reagent. The amount of bismuth present was estimated after precipitating the alkaloids with Dragendorff's reagent (Sreevidya and Mehrotra, 2003). Each experiment has three replicates and the experiment was repeated thrice.

Statistical analysis

Each experiment has three replicates and the experiment was repeated thrice and the data was subjected to one way ANOVA using Minitab version 15. A significance level of 0.05 was used for all statistical tests.

RESULTS

The percentage of extract after soxhlation was found to be 7.5% for *T. cardifolia* and 6.25% for *A. lanata* respectively. Preliminary phytochemical examination revealed the presence of steroids, tannins, saponins, flavonoids, phenols, alkaloids in both leaf and root extracts of *T. cardifolia* and *A. lanata*. Presences of phytochemical are the key candidate for the medicinal value of this plant.

Tab. 1. Preliminary screening of secondary metabolites in *T. cardifolia* and *A. lanata*

Name of the Phytochemicals	*T. cardifolia* (leaf)	*A. lanata* (leaf)
Steroids	+ve	+ve
Terpenoids	-ve	-ve
Fatty acids	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve
Anthocyanins	-ve	-ve
Leucoanthocyanins	+ve	-ve
Coumarins	-ve	-ve
Emodins	-ve	-ve
Flavonoids	+ve	+ve
Phenols	+ve	+ve
Alkaloids	+ve	+ve

Qualitative analysis showed high quantities of all the four secondary metabolites (Table 2). But the concentrations of alkaloids were high in both the plants (845.33±9.61) in *T. cardifolia* and *A. lanata*.

Table 2. Quantitative screening of phytochemicals (phenols, tannins, alkaloids, flavonoids) in methanolic leaf extract of *T. cardifolia* and *A. lanata*

S. No	Phytochemical	*T. cardifolia* *	*A. lanata**
1	Phenols (mg/gm)	172.33±2.52	142.00±2.61
2	Tannins (mg/gm)	186.67±5.21	159.67±2.08
3	Alkaloids (mg/gm)	520.33±9.61	490.00±5.00
4	Flavonoids (mg/gm)	169.33±8.33	128.67±5.03

* Each value represents mean±SD of three independent experiments and the values was significant at $p<0.05$.

DISCUSSION

The results of preliminary phytochemical screening of the crude methanol extracts of the two plants revealed the presence of all the constituents tested including; alkaloids, flavonoids, saponins, tannins, phenols, glycosides, anthraquinones, steroids, terpenes and fatty acids. These constituents are responsible for most pharmacological activities of plants (Abhimanyu Sharma 2010, Gagandeep Kaur *et al*., 2016). The multiple uses of this plant and its parts in treating various ailments was reported by several researchers (Sharma et al 2010, Ragavendran 2012). Keeping in view of the medicinal importance of these plants and based on literature the present study was initiated in order to know any unknown compounds in leaves of *T. cardifolia* and *A. lanata.* The quantitative analysis also revealed that the plants contain high amounts of phenols, tannins, flavonoids and alkaloids. The concentration of alkaloids are rich in *T. cordifolia when compare to A. lanata.* The presence of these phytochemicals are responsible for enhanced immune responses as was reported by Manjrekar (2000) and Dikshit (2000), anti-spasmodic, anti-pyretic, anti-allergic and anti-leprotic properties and anti-oxidant properties as reported by Asthana *et al* (2001) Nayampalli *et al* (1986), Ikram (1987) in *T. cordifolia*. Similarly the role of *A. lanata* in treating diseases was reported by Koperuncholan *et al*. (2010), Yamunadevi *et al*. (2011), Battu and Kumar (2012).

Phytochemicals are the non-nutrient compounds produced by the plants in self-defence to protect them from pest, microbes and environmental stress factors. Medicinal value of the any plant is depends on the phytochemical present within this plant. Herbs and plants contain diversified compounds with wide range of activities, which may be helpful in the development of new drugs (Shoeb, 2006; Menichini *et al*., 2009; Lei *et al*., 2011). Phytochemical screening of aqueous plant extracts showed the presence of steroids, terpenoids, fatty acids, tannins, saponins, flavonoids, phenols and alkaloids. The presence of phenolics, tannins, flavonoids and alkaloids in higher quantities in *T. cordifolia* and *A. lanata* indicates higher antioxidant potential and showed their effective role in several biological processes such as anticarcinogen, antiatherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). The results were agreed with the findings of Ofokansi *et al*. (2005) in leaf extracts of *Bryophyllum pinnatum* and *Ocimum gratissimum* (Ofokansi *et al*., 2005). Several workers reported that alkaloids and their synthetic drugs have analgesic (Antherden, 1969), antispasmodic and anti-bacterial properties (Okwu and Okwu, 2004).

Conclusion

The present study is conducted in order to know the status of various phytochemicals in *T. cordifolia* and *A. lanata* because about 30% of worldwide sale of drugs is based on natural products and knowledge about traditional indigenous medicine is important. Exploitation of pharmacological properties of the above two plants is needed and will be continued in further investigations.

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