



Qualitative and quantification analysis of phytochemicals from leaf aqueous extract of *Allamanda cathartica* L. and *Terminalia paniculata* Roth.

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ABSTRACT

Traditional systems of medicines are prepared from a single plant or combination of more than one plant. Phytochemical screening is responsible for medicinal activity of plant species. Hence in the present study the preliminary phytochemical screening and quantification of primary and secondary metabolites from aqueous leaf extract of *Allamanda cathartica* and *Terminalia paniculata* was carried out. The preliminary phytochemical screening of aqueous leaves extracts of *A. cathartica* and *T. paniculata* revealed that the presence of proteins, carbohydrates, reducing sugars, phenols, tannins, flavonoids, saponins, terpenoids, alkaloids, lignins, anthocyanins, steroids and glycosides; and absence of anthraquinones, leucoanthocyanins and fixed oils respectively. Quantification of phytochemical constituents from leaf of *A. cathartica* showed that the phenols was highest amount, followed by lipids, saponins, proteins, carbohydrates, flavonoids, tannins and alkaloids. Whereas in the leaves of *T. paniculata* showed that the phenols was highest amount, followed by alkaloids, saponins, lipids, proteins, flavonoids, carbohydrates and tannins. The results suggest that phytochemical properties for curing various ailments and possess potential antioxidant, anti-inflammatory, antimicrobial and leads to the isolation of new and novel compounds.

Key words: *Allamanda cathartica*, *Terminalia paniculata*, phytochemicals, leaves, aqueous extract

INTRODUCTION

Allamanda cathartica L. is widely growing perennial ornamental shrub belonging to the family Apocynaceae and also commonly known as the Yellow Bell, Golden Trumpet or The Buttercup flower. It can grow up to a height of 15 feet tall or more, is native to Brazil but widely cultivated throughout the world. The plant is used to relieve cough and made into decoctions for use as a purgative. This plant has anti-bacterial and anti-cancerous properties, it was also widely used in the treatment of jaundice. The root and stem of this plant contain two rare lactones which are active against polio virus and pathogenic fungi. Root is also used in various formulations to treat malarial symptoms. The leaves stem and branches of this plant are used against Snake bite^[1]. Moreover, all the plant parts are reported to be poisonous and hence the plant has not been extensively used in medicine. *Terminalia paniculata* Roth (Combretaceae) is a tropical tree with a large natural distribution in Western Ghats, India. *Terminalia paniculata* has been extensively utilized for wood and non wood products for construction and it often used to substitute teak. Other common uses of *Terminalia paniculata* wood are timber

for agricultural implements boat buildings, ply-wood, block boards and packing cases^[2]. Traditionally, flower juice and bark of *Terminalia paniculata* have been used as a remedy for cholera, for the treatment of inflamed parotid glands and in menstrual disorders^[3].

Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc;^[4]. Previously the crude drugs were identified by comparison only with the standard descriptions available, but recently due to advancement in the field of pharmacognosy various techniques have been following for the standardization of crude drugs^[5]. Medicinal herbs have been used in one form or another under indigenous systems of medicine. Dubey *et al.*^[6] mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. Number of plants were studied for qualitative and quantification of phytochemical for their medicinal values of *Svensonia hyderabadensis*^[7,8], *Nerium indicum*^[9], *Boswellia ovalifoliolata*^[5, 10], *Gloriosa superba*^[11], *Shorea tumbuggaia*^[12], *Ricinus communis* and *Euphorbia hirta*^[13], *Pongamia pinnata*^[14] and *Moringa oleifera*^[15].

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc^[16] i.e., any part of the plant may contain active

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components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers^[17-19]. In the present work, quantitative and quantification phytochemical analysis was carried in two important medicinal plants of *Allamanda cathartica* and *Terminalia paniculata*.

MATERIAL AND METHODS

Collection of Plant material

Fresh leaves of *A. cathartica* and *T. paniculata* were collected from the Tirumala hills and different locations of Chittoor district, Andhra Pradesh, India during the month of December, 2012. The leaves were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under shade after complete shade drying the plant material was used for phytochemical analysis.

Preparation of extract

The grinded leaf materials of 5gm weighed separately using an electronic balance and were crushed in 25 ml of sterile water, boiled at 50-60°C for 30 minutes on 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^[20].

Preliminary screening and quantification of phytochemicals

The condensed extracts were used for preliminary screening of phytochemicals such as steroids, alkaloids, lignin and phenols^[21]; glycosides, terpenoids and saponins^[22]; tannins, leucoanthocyanins^[23]; reducing sugars^[24]; anthraquinones^[25], flavonoids^[26]; proteins^[27], carbohydrates^[28], lipids^[29]; phenols and tannins^[30], flavonoids^[31], saponins and alkaloids^[20].

RESULTS AND DISCUSSION

Preliminary phytochemical screening of *Allamanda cathartica* and *Terminalia paniculata* showed that the leaves were rich in proteins, carbohydrates, reducing sugars, phenols, tannins, flavonoids, saponins, triterpenoids, alkaloids, lignins and anthocyanins, glycosides and steroids, absent of anthraquinone, leucoanthocyanins and fixed oils (**Table-1**).

Alkaloid compounds are found in aqueous leaf extracts of *A. cathartica* and *T. paniculata*. The highest levels of alkaloids were found to be maximum of *Terminalia paniculata* i.e. 0.300 ± 0.011 mg/gwt and lower in *Allamanda cathartica* i.e. 0.014 ± 0.001 mg/gwt. The alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structure types, biosynthetic pathways, and pharmacological activities^[32].

Maximum levels of carbohydrates were found in *Terminalia paniculata* i.e. 0.075 ± 0.030 mg/gwt and minimum levels of carbohydrates were found in *Allamanda cathartica* i.e. 0.061 ± 0.010 mg/gwt. Carbohydrates are one such group of carbon compounds, which are

Table-1: Preliminary phytochemical screening aqueous leaves extract of *Allamanda cathartica* and *Thespesia populnea*

S.No	Phytochemical components	Plant Name	
		<i>Allamanda cathartica</i>	<i>Terminalia Paniculata</i>
1.	Proteins	+	+
2.	Carbohydrates	+	+
3.	Reducing sugars	+	+
4.	Phenols	+	+
5.	Tannins	+	+
6.	Flavonoids	+	+
7.	Saponins	+	+
8.	Terpenoids	+	+
9.	Alkaloids	+	+
10.	Anthraquinone	-	-
11.	Lignins	+	+
12.	Anthocyanins	+	+
13.	Steroides	+	+
14.	Glycosides	+	+
15.	Leucoanthocyanins	-	-
16.	Fixed oils	-	-

Note: '+' indicates presence and '-' indicates absence

essential to life. Almost all organisms use carbohydrates as building blocks of cells and as a matter to fact, exploit their rich supply of potential energy to maintain life.

Flavonoids are present in *A. cathartica* and *T. paniculata*. The highest amount of flavonoid content was found to be maximum in *Terminalia paniculata* i.e. 0.077 ± 0.040 mg/gwt and minimum in *Allamanda cathartica* (0.056 ± 0.004 mg/gwt). Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent, the flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action of flavonoids are through scavenging or chelating process^[33]. They also inhibit microbes which are resistance to antibiotics. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity^[34] as, antioxidants, flavonoids provide anti-inflammatory action^[35].

Lipids are the supporters and storage molecule of cells. These are greasy materials which play important cellular structures. Lipids are being use by industry as highly stable lubricant and as renewable source of fuel. Lipids a diverse group of primary metabolites, includes reserve plant material such as fats, essential oils, waxes, terpenoids, and oleoresin, lipids are hydrophobic and a major components of cell membranes. Which act as vital cellular messengers and serving as module to hormones and vitamins with a strong foundation in research and development plant lipids have developed products that work with plant lipids with diverse requirements like culinary, medicinal and cosmetics^[36]. The highest amount of lipid content was found to be maximum in *Allamanda cathartica* i.e. 0.700 ± 0.057 mg/gwt and minimum in *Terminalia paniculata* i.e. 0.200 ± 0.057 mg/gwt.

Phenol compounds are present in *A. cathartica* and *T. paniculata*. Maximum levels of phenols were found in *Allamanda cathartica* i.e. 1.650 ± 0.087 mg/gwt and minimum levels of phenols were found in *Terminalia paniculata* i.e. 1.290 ± 0.045 mg/gwt. The higher amount of phenols is important in the regulation of plant growth, development and disease resistance. It can be used as fungicide, pesticides, antiseptic and disinfectant and in the manufacture of resins. Plant phenols may interfere with stages of Cancer process. Total levels of phenols were found to be higher in leaves of *Pongamia pinnata* [14]. Phenolics are secondary metabolites that are ubiquitously present in fruits. Many of the phenolics have been shown to contain higher levels of antioxidants activities. Fogliano et al. [37] have reported that plant phenols in red wine exerted cardio protective effect.

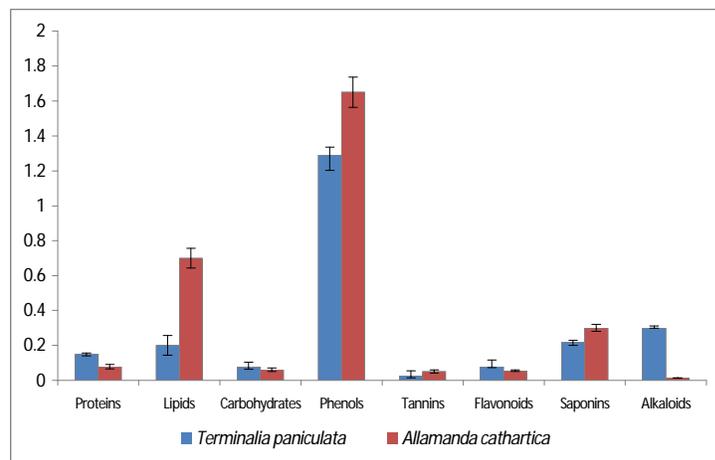
Total levels of proteins were found to be higher in *Terminalia paniculata* i.e. 0.151 ± 0.007 mg/gwt and lower *A. cathartica* i.e. 0.078 ± 0.012 mg/gwt (Table-2 and Graph-1). Proteins are primary components of living organisms. The presence of higher protein level in the plants points towards their possible increase in food value or that a protein based bioactive compound could also be isolated in future [38]. Proteins are essential to maintain the structure and function of all life, proteins are vital for the growth, repair, maintenance of muscles, blood, internal organs, skin hair and nails. After long hard workout, protein is essential to rebuild worn-out tissue and rebuild muscle.

Saponins are present in *A. cathartica* and *T. paniculata*. Maximum levels of saponins were present *Allamanda cathartica* i.e. 0.300 ± 0.020 mg/gwt and lower in *Terminalia paniculata* i.e., 0.220 ± 0.010 mg/gwt. Traditionally saponins have been extensively used as detergents as pesticides and molluscides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [39]. Saponin has relationship with sex hormones like oxytocin, oxytocin is a sexhormone involved in controlling the onset of labour pains in women and the subsequent release of milk [40], the presence of saponins in plants have been reported to be responsible for the toxic and stimulating activities observed in Chinese and Japanese medicinal herbs.

Table-2: Quantification analysis of phytochemical constituents from leaves of *Allamanda cathartica* and *Terminalia paniculata*

S.No	Phytochemical	Plant Name	
		<i>Allamanda cathartica</i> mg/gwt	<i>Terminalia paniculata</i> mg/gwt
1.	Proteins	0.078 ± 0.012	0.151 ± 0.007
2.	Lipids	0.700 ± 0.057	0.200 ± 0.057
3.	Carbohydrates	0.061 ± 0.010	0.075 ± 0.030
4.	Phenols	1.650 ± 0.087	1.290 ± 0.045
5.	Tannins	0.051 ± 0.010	0.026 ± 0.030
6.	Flavonoids	0.056 ± 0.004	0.077 ± 0.040
7.	Saponins	0.300 ± 0.020	0.220 ± 0.010
8.	Alkaloids	0.014 ± 0.001	0.300 ± 0.011

Note: \pm indicates mean standard deviation



Graph-1: Quantification analysis of phytochemical constituents from leaves of *Allamanda cathartica* and *Terminalia paniculata*

Tannins present in *A. cathartica* and *T. paniculata*. The total content of tannins were found to be maximum in *Allamanda cathartica*, i.e. 0.051 ± 0.010 mg/gwt and minimum in *Terminalia paniculata* i.e. 0.026 ± 0.030 mg/gwt. Tannins contribute property of astringency i.e. fasten the healing of wound and inflamed mucous membrane and have receives considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties. Tannins are complex moieties produced by majority of plants as protective substances; they have wide pharmacological activities and have been used since past as tanning agents and they possess astringent, anti diarrhoea, anti-inflammatory activities [41].

Glycoside substances found in *A. cathartica* and *T. paniculata*. Similar results are found in leaf extract of *Saraca indica* [42], *Andrographis neesiana* [43], *Calotropis gigantea*, *Ecbolium viride*, *Gmelina arborea*, *Jasminum grandiflorum*, *Morinda pubescens* and *Vitex negundo* [44], *Gomphrena serrata*, *Allamanda cathartica*, *Carissa spinarum*, *Caralluma attenuata*, *Chrysanthemum indicum* and *Nyctanthes arbortristis* [45]. Glycoside compounds are containing a carbohydrate and non-carbohydrate residue (moiety) in the same molecule. In these compounds, the carbohydrate moiety is attached by an acetyl linkage carbon-I to the non-carbohydrate residue (aglycone). They all contain steroid as aglycone component in combination with sugar molecules. They are important in medicine because of their action on heart and are used in cardiac insufficiency [46]. Thus, cardiac glycosides are drugs and can be used in the treatment of congestive heart failure and cardiac arrhythmia. They work by inhibiting the Na^+/Na^+ pump, resulting in an increase in the levels of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for concentration of the heart muscle, improves cardiac output and reduces distention of the heart [47]. However, some glycosides such as ovarian are toxic as it inhibits active transport of Na^+ in cardiac muscle (Sodium pump inhibitor), resulting in inhibition of translocases during electron transport chain and leading to death [48].

Lignins are present in leaf extracts of *A. cathartica* and *T. paniculata*. Similar results are found in leaves of *Abrus precatorius*, *Cissus quadrangularis*, *Elitoria ternatea*, *Dodonaea viscosa*, *Eucalyptus globulus*, *Kalanchoe laciniata*, *Mimosa pudica*, *Passiflora foetida*, *Quisqualis indica*, and *Terminalia arjuna*^[18]. Lignins are significant components in the global carbon cycle; the resistance of lignin to microbial degradation enhances its persistence in soils^[49].

Steroids are present in *A. cathartica* and *T. paniculata*. Steroids are present in the leaves of *Helichrysum longifolium*^[50], *Pterospermum canescens*^[51], *Carica papaya*, *Ocimum gratissimum*, *Adenia eissampeloids* and *Cymbopogon citratus*^[52], *Maytenus emarginata*^[53], leaf extract of *Dysophylla myosuroides* and tuber, leaf extract of *Talinum cuneifolium*^[54], root and leaves of *Echium pycnanthum*^[55]. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with sex hormones^[56]. Terepenoids are present in *A. cathartica* and *T. paniculata*. These are also found in the leaves of *Thespesia papulnia* and *Tridax procumbens*^[57], *Carica papaya*, *Ocimum gratissimum*, *Adenia cissampeloides* and *Cymbopogon citratus*^[58], *Andrographis echinoides*, *Alangium salvifolium*, *Cucumis melo*, *Tragia involucrata*, *Taxillus heyneanus*^[44]. Terpenoids which are attributed for analgesic and anti-inflammatory activities.

Anthocyanins are present in aqueous extract of *A. cathartica* and *T. paniculata*. Anthocyanins help the human immune system to work more efficiently to protect against viral infections. It is little bit more complex, specific types of anthocyanins may have a direct effect in decreasing influenza viruses infectivity by decreasing the ability of the virus itself to get into the human cell or to be related from infected cells or by having a viricide effect^[59].

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic can be attributed to their high phenols, tannins, triterpenoids, saponins and flavonoids. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation of techniques like extraction, purification, separation, crystallization and identification.

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