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Quantitative Estimation of Total Phenolic, Flavonoids, Tannin and Alkaloid Content of Aerial Part of Acalypha Indica Linn

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Abstract
Acalypha indicapresent study was to estimate the total phenolic, flavonoids, tannin and alkaloid content of aerial part. The total alkaloid content was recorded a s13.6 mg 100^{-1} total phenolics and tannin contenst were expressed as tannic acid equivalent and total flavonoids as rutin equivalent. The selected plant sample showed 72.1 mg TAE g^{-1} of total phenolics, 53.5 mg TAE g^{-1} of tannins and 24.9 mg RE g^{-1} of total flavonoids.

Keywords: *Acalypha indica,* flavonoids, tannin, phenolic, alkaloid.

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Introduction:

More than 4000 polyphenols (Flavonoids, monophenols and poly are found in vascular plants. Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents [1]. Flavonoids are the largest group of naturally occuring phenolic compounds, which occurs in different plant parts in free state and as glycosides[2]. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulacer, anti arthritic, anti angiogenic, anti cancer, protein kinase inhibition etc. the flavones and flavonols are the most widely distributed of all the phenolics[3]. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardio vascular disease, certain forms of cancer and age related degeneration of cell componetns. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals[4]. A variety of dietary plant flavonoids inhibits tumor development in experimental animal models[5]. The bi-flavonoids have the pharmacological eddects like their ability to inhibit the release of histamines, the adhesion of blood platelents and the action of lens aldose reductase, to block the inflammatory effects of hepatotoxins, and to act as a heart stimulant[6].

Materials and Methods

Determination of Total Phenolics and Tannins

Priciple

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to form a blue coloured complex and the colour intensity was measured at 725nm.

Procedure

10 ml of the extract was taken in a test tube and made up to the volume of 1ml with distilled water. Then 0.5ml of Folic-Ciocalteau reagent and 2.5ml of 20% sodium carbonate solution were added sequentially in each test tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40min. and the absorbance was recorded at 725nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents. Using the sample extract, the tannins were estimated after the treatment with Poly Vinyl Poly Pyrrolidine (PVPP). 100 mg of PVPP was weighed into a 110×12mm test tube and to this added 1ml of distilled water and 1ml of sample extract the content was vortexed and kept in the test tube at 4°C for 4 h. Then the sample was centrifuged at 3000 rpm for 10min. at room tempreature and the supernatant was collected. The supernatant has only phenolics whereas the tannins would have been precipitated along with PVPP. The phenolic content of

the supernatant was measured and expressed as the content of non-tannin phenolics ona dry matter basis. From the above results, the tannin content of the sample was calculated as follows

Tannin (%) = Total Phenolics (%)-Non-tannin Phenolics(%)

Determination of Total Flavonoid content

0.5 ml of aliquot of appropriately (10mg-12ml) diluted sample solution was mixed wit 2ml of distilled water and subsequently with 0.15 ml of 5% $NaNo_2$solution. After 6 min, 0.15 ml of 10% $AlCl_3$solutin was added and allowed to stand for 6min and then 2ml of 4% NaOH solution was added to the mixture was thoroughly mixed and allowed to stand for 15min. Absorbance of the mixture was determined at 510nm against water blank. The results were expressed as mg RE(Rutin Equivalent)g^{-1} of extract[8].

Results and discussion

The total alkaloids, total phenolics, total flaconoids and tannin contents of ethanolic extract of *A. indica* were determined and presented in Table. The total alkaloid content was recorded as 13.6 mg $100g^{-1}$. Total phenolics and tannin contents were expressed as Tannin acid equivalent and total flavonoids as Rutin equivalent. The selected plant sample showed 72.1 mg TAE g^{-1} of total phenolics, 53.5 mg TAE g^{-1} of tannins and 24.9 mg RE $g^{-1}$of total flavonoids. Medicinal plant based drugs have shown the added advantage of being simple, effective, free from side effects and offer a broad spectrum of activity with great emphasis on preventive action of chronic and degenerative diseases (*Chin et al.,* 2006). Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, flok medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (*Ncube et al.,* 2008; *Nirmala eta al.,* 2011 a,b). the medicinal plants have chemical substacnces called phytochemical that produce various physiological action on the human body. Phytochemical screening is an essential step towards the discovery of new drugs as it provides the informatin regarding the presence of particular primary and secondary metabolits in the plant extract of clinical significance. Phytochemicals derived from the plant sources are used for prevention and treatment of diabetes mellitus,cancer, heart diseases and high blood pressure (Waltner-Law *et al.,* 2002). The therapeutic effect of several medicinal plants haveben attributed to the presence of phenolic compounds such as flavonoids, phenolic acid, proanthocyanidins, diterpenes and tannins (Pourmorad*et al.,* 2006).

In the present study, the qualitative phytochemical analysis of ethanolic extract of *A. indica* revealed the presence of alkaloids, glycosides, flavonoids, saponins, phenols and tannins. The positive response of the above mentioned compounds in the ethanolic extract may be due to the dissolution capacity of phytochemiclas in the organic solvent. Earlier, similar studies were carried out in *Strumpfia maritima* (Hsu *et al.,* 1981), *Uncaria*species (Heitzman *et al.,*2005), *Mitracarpusscaber*(Abere *et al.,* 2007) and *Teucrium stocksianum*(Rahim *et al.,* 2012).

Natural products have played an important role in the development of drugs for various diseases. Until 1990's scientists thought that most of the compounds produced by the platns were useless waste products. These waste compounds are called as secondary metabolites. But later it was found that these compounds may perform a huge array of functions. Many of these compounds cannot be synthesized economically on commercial basis. The secondary metabolites have complex stereo structure with many chiral centres which may be essential for various biological activities[9]. The secondary metabolites from natural sources are good products for drug development because being elaborated within the living systems, they are perceive to exhibit more similaritites to drugs and show more biological friendliness than synthetic drugs[10]. Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines. Plant with natural products exhibit pharmacological and biological activities and play an important role in life-threatening conditions[11].

Flavonoids are present in all vascular plants and have been reported to exert multiple biological effects including anti-inflammatory, anti-ulcerogenic, anti-allergic, anti-viral and anti-cancerous activities[12,13]. Tannins have been reported in the leaves of Pomegranate, Tambolan and Guava and medicinally rannins are used in anti-diarrhoeal and anti-haemorrhoidal preparations[14,15]. Saponins are glycosiders of steroids, steroid alkaloids found in plants, especially in plant skins where they form a waxy protective coating. They are useful in lowering cholesterol, as antioxidants and anti-inflammatory agents.

Terpenoids are diverse class of naturally occurring organic chemicals found in all classes of living organisms, which may exhibit antibacterial properties[16]. Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia and are found as secondary metabolites in several plants like *Digitalis, convallaris*and *Euphorbia* Species Alkaloids are the largest class of secondary plant substances present in higher plants of some families viz., Papaveraceae, Liliaceae, Solanaceae, Rubiaceae, Rutaceae, Boraginaceae and Asclepiadaceae and are reported to possess defensive effects[17]. Alkaloids are stored predominantly in actively growing young tissues, roots, stem barks, flowers and seedlings. In the present study, the ethanolic extract of *A. indica* was reported to contain 13.6 mg 100^{-1} of alkaloids (Table 1). Presence of alkaloids was reported in *Cephaelis*species[18]. *Anthoxephaluscadamba*and *cinchona* spa[20].

Phenolics are the most important secondary metabolites present in plant kingdom. These diverse group of compounds serve as potential natural antioxidant in terms of the ability to act as both efficient radical scavenger and as metal chelator. It has been reported that the antioxidant activity of phenols is mainly due to their ability to act as hydrogen donor, singlet oxygen quencher and their redox property[21]. In the present study, the total phenolics content of ethanolic extract of *A. indica* was estimated as 72.1 mg^{-1} extract (Table 1).

Tannins are water soluble plant polyphenolics which cause protein precipitaioj from aqueous solutions, located in vacuoles[22]. This group of compound has received a great deal of attention in recent years, since it was suggested that the consumption of tannin containing beverages especially green teas and red wines can cure or prevent a variety of illness. Tannins are complex moieties produced by most of the plants as protective agent with wide pharmacological avtivities. They have been shown to possess astringet, anti-inflammataory, antidiarrhoel, antioxidant and antimicrobial properties[23].

In the present study, the tannin content of ethanolic extract of *A. indica* was recorded as 53.5 mg TAE g^{-1} (Table 1). This is in line with the report of kanna*et al.*[24]. Flavonoids are well known phytochemicals having the biological effects such as free radical svacenging, modulation of enzymatic activity, antibiotic and anti-inflammatory activities. It is reported that flavonoids are natural products which are shown to exhibit biological properties related to antioxidant activity[25]. The flavonoid content of the present study plant was found to be 24.9 mg Reg^{-1} (Table 1). This correlates with the report of Lopes *et al.*[26] in *Chiococcabraquiata.*

The findings of the present study indicated that the selected plant sample was reported to contain alkaloids, phenloids, phenolic compounds, tannins and flavonoids which may contribute to the antioxidant activity of the etanolic extract of *A. indica.* This result correlates well with the findings of Ashafa*et al* [27], who reported that the leaf extract of *Felicia muricata* had shown significant antioxidant activity due to the presence of secondary metabolites such as alkaloids, flavonoids and phenols.

Table-1 Quantitative analysis of phytochemicals in ethanolic extract of *A. indica*

Constituents	**Concentration**	**Unit**
Total Alkaloids	13.6 ± 3.4	mg $100g^{-1}$
Toral Phenolics	72.1 ± 2.8	mg $TAEg^{-1}$ extract
Tannins	53.5 ± 4.2	mg $TAEg^{-1}$ extract
Total Flavonoids	24.9 ± 9.1	mg Reg^{-1}extract

Values are means of three independent analyses of the extract; ± standard deviation (n=3)
TAE - Tannic acid equivalent
RE - Rutin equivalent

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