Phytochemical Screening and Antioxidant Activity of Tuber Extracts of *Tacca pinnatifida* J.R.&J.G.Forst

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Research Article

Abstract: The Western Ghats of India are known to be a major biological hotspot that supports plant diversity and endemism. Members of the Taccaceae are famous for their use as medicinal herbs. *Tacca pinnatifida* J.R&J.G.Forst is erect perennial herb occur as undergrowth in moist shady places in forests of Maharashtra.The phytochemical study and antioxidant activity of the tuber extracts of *Tacca pinnatifida* J.R&J.G.Forst were evaluated. Phytochemical screening indicated that, tubers are rich in a variety of primary and secondary metabolites such as carbohydrates, Alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins and volatile oils. This piece of work highlights the biochemical and ethno pharmacological significance of *Tacca pinnatifida* J.R&J.G.Forst.

Keywords: *Tacca pinnatifida* J.R&J.G.Forst, Phytochemicals, Antioxidants, Medicinal plants.

Introduction

Tacca pinatifida J.R&J.G.Forst. is the only genus in the family Taccaceae, a newly-developed plant family carved out of the Dioscoreaceae, but both families still share a close taxonomic relationship (Caddick R.L.,2002). The plant is native to Malaysia and the Pacific Islands (Purseglove J.W. 1972, Kay D.E. ,1982). and it is naturally distributed from Western Africa, through Southern Asia to northern Australia. Tuberous marshy monsoon perennial, flowering in August – September found in shades of moist deciduous forest in peninsular part of India.(Cook,1903,Mulla R.M.,1993). Leaves are large and deeply divided, 30 to 70 cm long and up to 120 cm in width. The leaf upper surface has depressed veins, and the under surface is shiny with bold yellow veins. Flowers are borne on tall stalks in greenish-purple clusters, with long trailing bracts. The plant is usually dormant for part of the year and dies down to the ground. Later, new leaves will arise from the round underground tuber. The tubers are hard and potato-like, with a brown skin and white interior.(Wagner et el,1990). The plants are cultivated in Travancore for tubers and foliage. The tubers used medicinally and source of starch. (Okwu,2004,Mulla,R.M.1991).The fresh tubers are acrid, bitter, poisonious and used for treatment of piles. The macerated fresh tubers ,repeatedly washed and salt water yield a nutritive starch of excellent culinary properties used to prepare porridages, cakes and other meats, can also no used as laundry starch. The bitter extract preserved by washing and grated tubers in running water is a rubefacient and is also given diarrhoea and dysentery. The flour from tuber has following composition water 18.0%,fiber 0.05%,total nitrogen 0.01%,ether extractives 3.0% and starch 76.0%.The presence of β – sitosterol, ceryl alcohol and taccallin 0.003%,which also gives positive tests for alkaloids.(Watt,1893,Kirtikar and Basu, 1937, Peter, *et al.* 1960, Scheur, *et al.* 1963). Phytochemicals are chemical compounds formed during the plants' normal metabolically processes(Okigbo , 2009).These chemicals are often referred to as secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids. most of these phytochemicals are produced through biosynthesis in the metabolic pathways.(Heldt , 2005). In traditional Hawaiian medicine the raw tuber was mixed with water and red clay and consumed to treat diarrhea and dysentery. This combination was also used to stop internal hemorrhaging in the stomach and colon and aplied to wounds to stop bleeding. (Krauss, Beatrice H. 1979). In traditional Hawaii medicine, the raw tubers are mixed with water and red clay and consumed to treat diarrhea and dysentery, as well as to stop stomach hemorrhage. In Ivory Coast, a leaf decoction is taken orally for scrotal elephantiasis and for oedema of the stomach (Khasim et.al,1999). The bitter raw tubers are used to treat stomach ailments, mainly diarrhea and dysentery in many Polynesian Islands (Kay D.E.,1987, Brand-Miller J.,1993).The root starch is used to stiffen fabrics in some of the Islands [14]. In traditional Hawaii medicine, the raw tubers are mixed with water and red

clay and consumed to treat diarrhea and dysentery, as well as to stop stomach hemorrhage. In Ivory Coast, a leaf decoction is taken orally for scrotal elephantiasis and for oedema of the stomach(Ukpabi U.J., 2009).

Figure 1: Habit of *Tacca pinatifida.* ***Figure 2:*** Tuber of *Tacca pinatifida.*

2. Materials and Methods

2.1 Sampling

Fresh samples of tubers of *Tacca pinatifida* Forst were collected from Uttan region of Western Ghats of Maharashtra (Figure 1 and 2).Fresh tubers were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The shade dried material was ground into coarse powder using mechanical grinder(Panasonic make). This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature till further phytochemical screening of secondary metabolites.

2.2 Soxhlet Extraction

Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus with accurately weighed 10 g of the drug powder for 18-40 h. Extraction was performed with water, methanol, chloroform, acetone and IPA as the extracting solvent. The extraction was conducted for 6-8 h/day and finally all the extracts were evaporated under vacuum. The water, methanol, chloroform, acetone and IPA extracts of tubers of these plants were prepared according to standard methods (Harbone, 1998). These extracts were sealed in airtight containers and stored at -4^0C.

2.3 Phytochemical Screening

Phytochemical screening of active plant extracts was done by standard tests for alkaloids, carbohydrate, glycosides, saponins, flavonoids and phenols (Khandelwal.2000) which could be responsible for antioxidant activity (Table 1).

2.3.1 Antiscavaging activity

DPPH solution (0.1 mM) was prepared in methanol by dissolving 0.0394 gm DPPH in 1000 ml methanol. The solution was kept in darkness for 30 minutes to complete the reaction. The free radicles scavaging activity of the crude extracts was determined by the 1,1-diphenyl-2-picryl-hydrazil (DPPH). The antioxidant activity was measured by (Brand Willium *et al.* 1995) method.Where in the bleaching rate of stable free radicle, DPPH was monitoried at a characterstic wavelength in the presence of the sample. In its radical form, DPPH absorbed at 570 nm, but upon reduction by an antioxidant or radical species its absorption decreased. The capability to scavange the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%)= ($ABS_{control}$- ABS_{sample})/($ABS_{control}$)X 100), where as $ABS_{control}$ is absorbance of negative control and ABS_{sample} is the absorbance of the reaction mixture containing the sample extract.

2.3.2 Mineral analysis

Micro-scaled digestion

CEM-MARS 6 microwave oven was used for micro-scaled digestion. 0.5 gms of herbal samples were weighed and transferred to CEM- Xpress vessels. 8-10ml of conc HNO_3 was added to the samples. The samples were pre digested for 10-15 minutes prior to capping the vessels. The CEM- Xpress vessels were assembled for microwave irradiation. The microwave program was adjusted with respect to the number of vessels and reference to the guidelines of CEM at 1000W with 100% level. A 25 minutes ramping period was used to reach the digestion temperature of 180^0C which thereupon was maintained for 15 minutes. The CEM- Xpress vessels were kept in fume hood for cooling and to release the pressure by uncapping . The contents were transferred to 50 mL volumetric flasks and volume were made with distilled water. The solutions were filtered prior to use. Microwave digestion is a common technique used by elemental scientists to dissolve heavy metals in the presence of organic molecules prior to analysis by inductively coupled plasma, atomic absorption, or atomic emission measurements.(Kingston,H.M.,*et al.* 1988). ICP mineral analysis: Diluted samples were used for further analysis by using Teledyne Leeman, ICP (Induction Coupled Plasma). It hs been shown that ICP MS has higher sensitivity and lower instrumental limits than other rapid multielemental techniques.(Jarvis,L1992)

2.4.3 Vitamin E analysis by HPLC

Standard preparation

A standard dl □-tocopherol acetate (96%) (Vitamin E) manufactured by Merck was used for calibration of standard curves. 1mg of dl □-tocopherol acetate was dissolved in 1mL in HPLC grade methanol. The dilutions of 100, 50, 25, 10 µg/mL was prepared. The pre-treated sample extracts and stock solutions were filtered through 0.45-µm syringe filters.

2.4.4. Vitamin C

2,6-dichlorophenol-indophenol sodium salt (DCPI): 0.025% ethanolic solution of DCPI was prepared for the detection of Vitamin C. To the 0.5mL of sample extracts, 2 drops of DCPI indicator was added. The blue coloration changed to red confirmed the presence of

vitamin C. The test was carried out for all the extracts(British Nutrition Foudation,2004).

3. Result and Discussion

3.1. Optimisation of extraction method

In order to extract the phytochemicals from herbal samples efficiently,variables involved in this procedure were optimised,including extraction solvent (Water, Methanol, Chloroform, Acetone, IPA, 100%),extraction method (Soxhlet, reflux, percolation), and extraction time (18-40 hr). The extraction time in water was 40 hr. The biomass was refluxed for 40hrs,then it was dried naturally for 2-3 days. To the dried biomass 100% methanol was added and the reaction was percolated for phytochemicals. The methanolic fraction was collected in amber colored bottle under nitrogen atmosphere. The material was dried for 5-6 hrs. The procedure was repeated for chloroform, acetone and IPA. The extraction time was optimized for all the samples. All the extract was preserved under nitrogen atmosphere in amber colored bottle.

3.2. Phytochemical Screening

Pytochemical screening of the tuber extracts of *Tacca pinatifida* revealed the presence of different phytochemicals. Indeed phytochemical investigations have resulted in occurrences of carbohydrates, alkaloids, glycosides, saponins, flavanoids, phenols, Vitamin E and Vitamin C. Table 1 illustrates the results of phytochemical screening of all the extracts of *Tacca pinatifida.* Alkaloids, comprising a large group of nitrogenous compounds are widely used as therapeutic agents in the management of cancer (Chabner B.A,1990). Saponins are glycosides of both triterpenes and steroids having hypotensive and cardiac depressant properties, and have been detected in over seventy plant families(Basu et.al.1967, Olaleye M.T. 2007).They have been shown to possess beneficial properties by lowering the cholesterol level, have anti-diabetic and anticarcinogenic properties as well as being used as an expectorant and emulsifying agent(Edeoga H.O,2006). Saponins are reported as a major component acting as antifungal secondary metabolite(Onwuliri F.E.,2003).However, environmental factors have been identified as responsible for changes and determination of the secondary metabolites in a plant(Waterman,P.G.1989). As such, same plant from different environments could have different phytochemical content. Previously,(Randrianalijaona *et al.*,2005) reported the seasonal changes in the chemical composition of essential oils in more than seventy *L. camara* from different parts of the world. Similarly,(Bhakta & Ganjewala,2009) reported ontogenic variation in secondary metabolites such as phenolics, anthocyanins, and proanthocyanidins in *L. camara*. In addition, (Fonseca, et. al.,2006) confirmed the fluctuation of secondary metabolite contents in medicinal plants with changing environment.

Table 1: Preliminary phytochemical screening of active plant tuber extracts

Constituents	Test	Observation	*Tacca pinatifida*				
			S1 W	***S2 M***	***S3 C***	***S4 A***	***S5 IP***
Carbohydrates	Benedicts Reagent	Red precipitate	+	+	+	+	+
Alkaloids	Mayer's Reagent	White precipitate	-	+	-	-	-
Glycosides	Borntranger's Reagent	Pink coloration	+	+	+	+	+
Saponins	Foaming	Frothing persisted for 10-15 min	+	+	-	-	-
Flavonoids	Shinoda	Pink-Red colouration	+	-	-	-	-
Phenols	Ferric chloride	Dark brown coloration	-	+	+	+	+
Vitamin C	2,6-dichlorophenol-indophenol sodium salt	Red Coloration	+	-	-	-	-
Vitamin E	HPLC method		-	-	+	-	-

S1=Water, S2=Methanol, S3=Acetone, S4=Chloroform, S5=Isopropyl acetate

3.3Antiscavaging activity

The phytochemical screening of the crude tuber extracts showed the positive reactions for alkaloids,flavonoids,phenolic compounds,saponins, glycosides, carbohydrates, Vitamin C, Vitamin E and Minerals. The scavenging ability assayed is the ability of extracts to react rapidly with DPPH radicals and reduce most DPPH radical molecules. The antioxidant capacity

tuber extracts was measured by DPPH anti scavenging activity method and the results were expressed in table 2. The DPPH anti scavenging activity of aqueous extract was 33.57 %, However, the value of methanolic extract (76.53%) which is 4 fold higher than chloroform and acetone extracts.(Figure no.3). The higher anti scavenging activity in methanolic extract shows that a more reducing characteristics. The methanolic extract displayed significant antioxidant activity. These results might suggest higher medicinal suitability of alcoholic extracts in various antioxidant applications. The evaluation of secondary metabolites in four Nigerian native plants; *Cissampelos owarensis, Tacca leontopetaloides, Euphobia hirta* and *Euphobia thymifolia*. Reveals the presence of alkaloids, tannins, saponins, triterpenes, flavonoids, glycosides and carbohydrates. *Cissampelos owarensis* contains all the metabolites while *Tacca leontopetaloides* contain all the metabolites except flavonoids,the plants have reducing characteristics hence can act as good antioxidants.(Habila, J.D,2011).

Table 2: Anti-Scavenging(DPPH) Activity

Sr.No.	Species Name	Code	Extract	Anti-Scavenging(DPPH) Activity (%)
1	***Tacca pinatifida***	**BX-3**	Water	34.57
			Methanol	76.53
			Chloroform	21.75
			Acetone	17.97

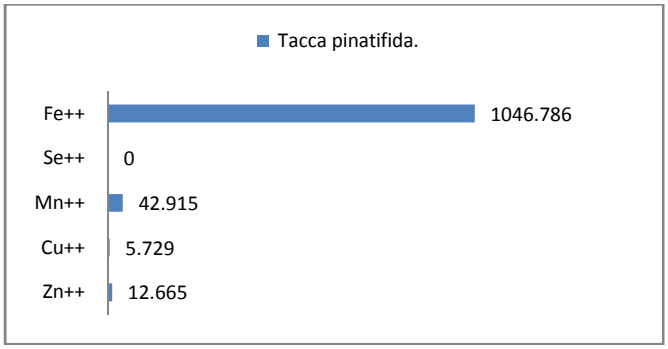

Figure 3: Mineral concentration in tuber extracts of *Tacca pinatifida*

3.4 Mineral analysis:Optimization and calibration for of *Tacca pinatifida* tuber extracts

After optimization a new calibration method was created for measuring these samples, the wavelengths used for calibration were Cu 324.754 nm, Mn 257.610, Se 196.090, Fe 259.940, and Zn 213.856 (Table 3). Calibration STD solutions were measured 3 times one by one with an RSD < 1%. Once all the calibration standards are finished, a necessary back ground correction was applied for each wavelength. The samples were measured thereafter with 3 repro. The average sum of the 3 measurements is tabulated in the analysis report. Quantitative multi-elemental analysis by inductively coupled plasma (ICP) spectrometry depends on a complete digestion of solid samples. However, fast and thorough sample digestion is a challenging analytical task which constitutes a bottleneck in modern multi elemental analysis. Additional obstacles may be that sample quantities are limited and elemental concentrations low. In such cases, digestion in small volumes with minimum dilution and contamination is required in order to obtain high accuracy data. We have developed a micro-scaled microwave digestion procedure and optimized it for accurate elemental profiling of plant materials. A commercially available 40- position rotor with 5 ml Poly tetra flouro ethylene (PTFE) vials, originally designed for microwave-based parallel organic synthesis, was used as a platform for the digestion. The novel micro-scaled method was successfully validated by the use of various certified reference materials (CRM). The micro-scaled digestion procedure was applied on crude powder of dried plant material in small batches. The contents were transferred to 50 mL volumetric flasks and volume was made with distilled water. The solutions were filtered prior to use. Teledyne Leeman, ICP spectrometer was calibrated by using Leeman standard, National Institute of Standards and Technology (NIST), USA. Diluted samples were used for further analysis. Iron and copper are of great importance for life. As redox-active metal they are involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis.

Manganese is essential for plant metabolism and development and occurs in oxidation states II, III, and IV in approximately 35 enzymes of a plant cell. Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of biomembranes. Most of the zinc enzymes are involved in regulation of DNA-transcription, RNA-processing, and translation. Although the essentiality of Se to plants has not been established yet,

Se is considered a beneficial element in promoting plant growth in some plant species. We have determined the five elements in coarse tuber powder of *Tacca pinatifida* are given in Table 4. Thereby, the concentration of minerals in tuber had the different profiles and quantitative differences had been detected.The most abundant microelement was Fe, where as copper was found at the lowest concentration. The content of Iron was especially high in comparison to Zn, Cu,and Mn. Selenium is undetected. Mineral analysis showed that major mineral contribution of Iron (1046.786 ppm) followed by Manganese (42.915 ppm), Zinc (12.665 ppm) and Copper (5.729 ppm) (Figure no.4). Vitamin E, vitamin C, carotenoids, Se and other trace minerals are important antioxidant components of animal diets and their roles in animal health and immune function are indispensable. In addition, several metalloenzymes which include glutathione peroxidase (Se), catalase(Fe), and superoxide dismutase (Cu, Zn, and Mn) are also critical in protecting the internal cellular constituents from oxidative damage. Only when these metals are delivered in the diet in sufficient amounts can the animal body synthesize these antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and damage to biological molecules and membranes. (Lee R. McDowell,2007).

Table 3: Instrumental characteristics and setting for ICP-OES:Spectrometer LEEMAN LAB's Simultaneous ICP-OES PRODIGY XPDual System

	Parameters Range		Actual Parameters
	Min	Max	
Power	0.1	2.0	1.1 KW
Coolant Flow	5	20	18 L/Min
Auxiliary Flow	0.0	2.0	0.2 L/M
Nebulizer Flow	5	60	34 psi
Plasma Torch	--	--	Dual
Spray Chamber	--	--	Cyclonic
Nebulizer	--	--	Concentric
Sample Aspiration Rate	0.5	2.0	1.4mL/min
Replicate read time	--	--	40 sec per replicate for Axial

Table 4: Accuracy of elemental concentrations in *Tacca pinatifida* after micro-scaled digestion

Species	**Zn^{++}**	**Cu^{++}**	**Mn^{++}**	**Se^{++}**	**Fe^{++}**
Tacca pinatifida.	**12.665**	**5.729**	**42.915**	**00**	**1046.786**

3.5. Qualitative analysis of Vit E by HPLC

A .Optimisation of HPLC method

To meet the requirements for quantitative analysis,the following HPLC parameters were examined, including different columns (Agilent SB-C18 length 250mm and 150mm, width 4.6, particle size 5um), column temperature (25^0C), and UV wavelength(220nm). The best chromatographic resolution was obtained on Agilent SB-C18 length 4.6 X 150mm, 5um column at 25^0C. The UV detector was monitored at 200-380 nm for finger printing analysis because the peaks were observed under this wavelength. The high intence peak was observed at 220nm.The tuber extract of Chloroform (S3) shows presence of Vitamin E. It is also added to foods like cereals. Most people get enough vitamin E from the foods they eat. People with certain disorders, such as liver diseases, cystic fibrosis, and Crohn's disease may need extra vitamin E. High doses of vitamin E may increase risks of prostate cancer and one type of stroke. Methanol extract of root of *Lucas aspera* possessed antioxidant activity near the range of vitamin E and thus could be a potential rich source of natural antioxidant.(Chew,A.L.2012). Tocopherols and tocotrienols, collectively known as tocols, are amphipathic and lipid-soluble compounds that are easily oxidized when subjected to heat, light and alkaline conditions (Kamal-Eldin, 1996, Eitenmiller, R.R, 2004). HPLC is most widely used technique to analyze tocols, and both normal-phase (NP) and reversed-phase (RP) chromatography are applied.(Abidi, S.L. ,2000, Ruperez, F. J., 2001,Kamal-Eldin, *et al.*,2000).Tocols are stable under HPLC conditions, easy to dissolve in appropriate solvents, and there are several detectors that can be combined with HPLC to detect tocols. Vitamin E functions as a chain-breaking antioxidant, neutralizing freeradicals and preventing oxidation of lipids within membranes (McDowell, 2000).

B. Method validation and calibration

To obtain the calibration curve, working solutions of four concentrations containing vit-E was analysed in triplicate. The calibration curves were established by plotting peak areas versus the concentration of each analyte. In the regression equation y = ax + b, x refers to the concentration of pure dl □-tocopherol acetate (lg/mL), y the peak area, and r the correlation coefficient.

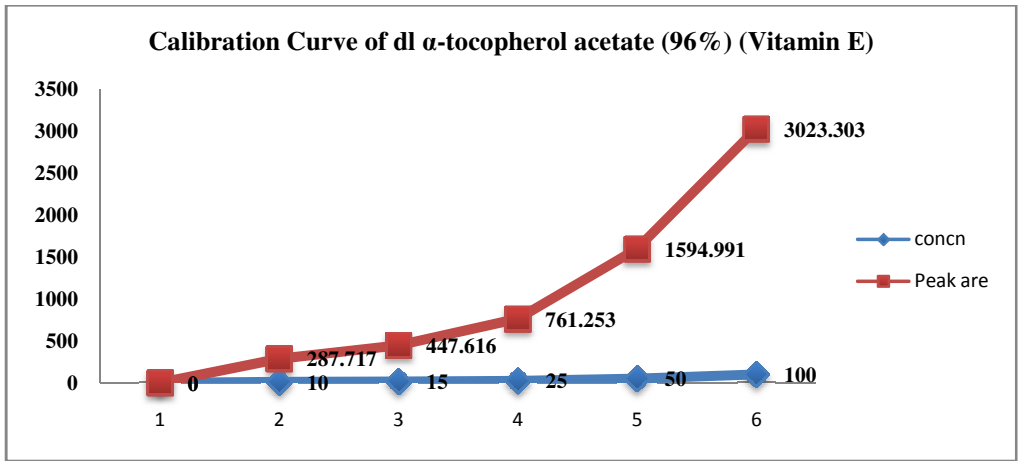

Figure 4: Calibration Curve of dl α-tocopherol acetate (96%) (Vitamin E)

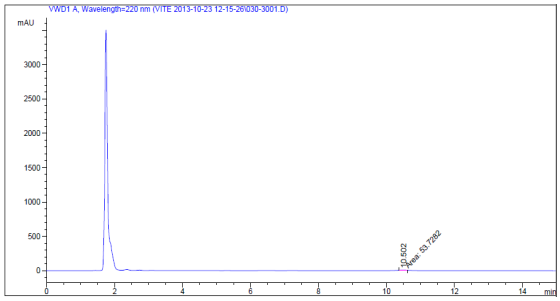

Figure 5: *Tacca pinatifida* tuber contain Vit.E in Chloroform extract

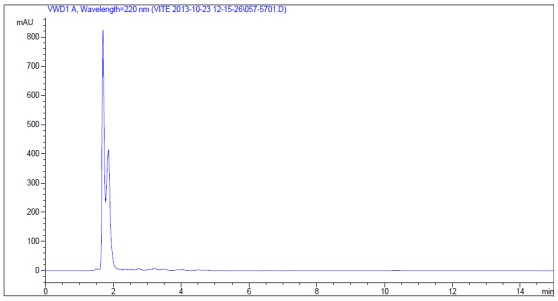

Figure 6: *Tacca pinatifida* tuber contain Vit.E in Methanolic extract.

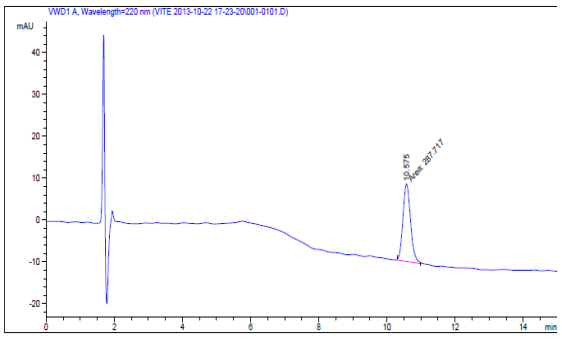

Figure 7:.Standard graph at 10 ul Vitamin E acetate.

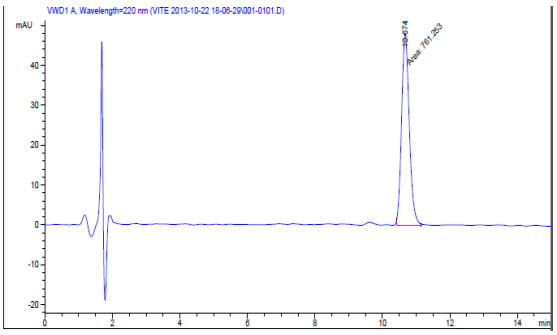

Figure 8: Standard graph at 25 ul Vitamin E acetate

Table 5: Vitamins and minerals in antioxidant systems (a)(Weiss,2005)

Nutrients	Component (location in cell)	Function
Vitamin C	Ascorbic acid (cytosol)	Reacts with several types of ROS/RNS
Vitamin E	alpha-tocopherol (membranes)	Breaks fatty acid peroxidation chain reactions
beta-carotene	beta-carotene (membranes)	Prevents initiation of fatty acids peroxidation chain reaction
Selenium	Glutathione peroxidase (cytosol)	An enzyme that converts hydrogen peroxide to water
Copper and zinc	Superoxide dismutase (cytosol)	An enzyme that converts superoxide to hydrogen peroxide
Manganese and zinc	Superoxide dismutase (mitochondria)	An enzyme that converts superoxide to hydrogen peroxide
Copper	Ceruloplasmin (water phase)	An antioxidant protein, may prevent copper and iron from participating in oxidation reactions
Iron	Catalase (cytosol)	An enzyme (primarily in liver) that converts hydrogen peroxide to water

Conclusion

Tacca pinatifida have an ancient history of the multiple indigenous uses and is one of the most highly commercialized indigenous traditional medicine from India.Investigation of the phytochemicals and their biological activity have provided scientific support for many of its traditional uses. An improved RP-HPLC-UV-method has successfully applied for determination of dl α-tocopherol acetate in organic extracts of *Tacca pinatifida.* Similarly the results obtained from phytochemical analysis illustrates the occurrences of various micronutrients ie carbohydrates, vitamine C, vitamine E, flavonoids, phenols, glycosides, saponins and minerals ie Zn, Cu, Mn, Fe. The present findings for microelements and minerals suggested that their contents are responsible for significant antioxidant activity in all extracts.

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