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ANALYSIS OF STEM OF *TINOSPORA CORDIFOLIA*, LEAVES OF *ANDROGRAPHIS PANICULATA* AND ROOT AND LEAVES OF *BOERHAAVIA DIFFUSA* FOR NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION

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ABSTRACT

Medicinal plants found diverse use in the society from medicine to cosmetics and herbal foods as they have vast potential for their curative medicinal uses. In view of this, the fresh stem of *Tinospora cordifolia*, leaves of *Andrographis paniculata* and roots and leaves of *Boerhaavia diffusa* were evaluated for nutritional and phytochemical compositions. The results showed that the leaves were found to be richer in vitamin C and minerals as compared to stem and root parts, while stem and root samples were found to be rich in fibre content. Berberine content (0.03294 %) in *T. cordifolia* and andrographolide (1.8 %) in *A. paniculata* were found to be high. The leaves of *B. diffusa* were found to be richer in percent radical scavenging activity (24.32 %) as compared to *T. cordifolia* stem (21.72 %) and *A. paniculata* leaves (23.56 %). *B. diffusa* leaves were found to be richest source of phytochemicals and percent radical scavenging activity among the parts analyzed, except saponins. The nutrient compositions obtained from the analysis suggested that these plant parts are a good source of antioxidants, vitamin and macro-and micro-nutrients make them strongly suitable to be incorporated into human nutrition.

Keywords: *Boerhaavia diffusa*, *Andrographis paniculata*, proximate and chemical compositions.

INTRODUCTION

Medicinal plants have been in major demand due to the great efficiency of herbal remedies. The medicinal plants have been prized for their medicinal flavouring and aromatic values. Today, medicinal plants are finding diverse use in the society from medicine to cosmetics, herbal drinks, herbal foods and other articles in the daily uses. Herbs play a significant role especially in modern times. The movement is based on the belief that the plants have a vast potential for their curative medicinal uses as herbal food products (Goyal, 2008, Rajakumar, 2014). Herbs of similar, supporting or enhancing nature are supposed to be added to the food products for intensifying the medicinal effects (Hasler, 2005).

The beneficial medicinal effects of plant materials typically results from the combinations of secondary metabolites such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins, fatty acids and gums (Okwu DE, 1999, Saikia, 2013 and Okwu DE, 2001). Vegetable is basically any part of a plant, be it leaves, roots, seeds or fruits that can be eaten. This food of plant origin contains many bioactive compounds and thus serves as an important source of minerals, vitamins and certain hormone precursors in addition to protein and energy sources (Cho E, *et.al*, 2004). Leafy vegetables have continued to provide populations with limited access to meat or fish, a rich source of proteins and micronutrients essential for pregnant and lactating mothers, as well as young and growing children (Penny MK, *et.al*, 2004). Studies have shown that vegetarians are less susceptible to diseases, live longer, healthier and more productive lives with stronger immunity (Akindahunsi AA, *et.al*, 2005, Mogra, 2013). However, there has been a reduction in the consumption of vegetables with each passing decade (Aranceta J, 2004). *Tinospora cordifolia*, *Andrographis paniculata* and *Boerhaavia diffusa* are wide species of vegetables that are relatively underutilized and in most cases neglected (Keary, 1985). The leaves are cooked and eaten as vegetable. Domestic livestock also grazes the plant especially during famine. *B. diffusa*, commonly called hog weed, is known as erimmirii (which literally means water-food) by the Ibos of southeastern Nigeria. The leaves are cooked and eaten as vegetable. The plant is used in folkloric medicine to treat convulsions and as a mild laxative and febrifuge (Adesina SK, 1979). The roots and leaves are considered to have an expectorant action, to be emetic and diuretic in large doses and are used in the treatment of asthma. The thick roots, softened by boiling

are applied as a poultice to draw abscesses and to encourage the extraction of guinea worm (Keary, 1985). The *Boerhaavia diffusa* (sant, thikri) is an herb known for its antioxidant, hepatoprotective and antistress activity. The active compounds in leaves and root are antioxidants, boeravinone, repenone and punarnavoside (Sastry, 2008). *Andrographis paniculata* (Kalmegha) is herb and known for its hepatoprotective, antihepatitis B and anticancer activity. The active compounds in leaves are andrographolides, kalmeghin, and andrographin. The leaves contain the highest amount of andrographolide, the most medicinally active phytochemical in the plant, while the seeds contain the lowest. The primary medicinal component of Andrographis is andrographolide. It has a very bitter taste, is a colorless crystalline in appearance, and is called a "diterpene lactone" - a chemical name that describes its ring like structure (Sastry, 2008). *Tinospora cordifolia* (Guduchi) is one of the extensively used herb and famous for its hepatoprotective activity, antioxidant, antidiabetic, anti-inflammatory, antiaging, and memory enhancing properties. The active adaptogenic constituents in stem, root and leaves are alkaloid berberine, giloin and diterpene compounds (Singh *et al.*, 2003).

The neglection of these vegetables coupled to the growing reduction in consumption of vegetables and evaluating the levels of some macro and micronutrients of *T. cordifolia, B. diffusa* and *A.paniculata* prompted this study so that this study will increase interest in them.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF PLANT MATERIALS

Plants parts (stems, leaves & roots) of three herbs (*Tinospora cordifolia*, *Andrographis paniculata*, *Boerhaavia diffusa*) were collected from a Roxberg herbal garden of Botany Department, University of Allahabad from the plant stalk and were cleaned by water and immediately analysed for proximate, vitamin & mineral content and phytochemicals content.

PROXIMATE ANALYSIS

Proximate observations for normalized samples of plant parts of *T. cordifolia*, *A. paniculata* and *B. diffusa* were estimated in triplicate for moisture, fat, protein, ash crude fibre contents and carbohydrates. These were determined by methods as described by AOAC, (2005) and total ash and mineral content as given by Rangana, (2005). Carbohydrate content was determined by difference method. All determinations were done in triplicates.

ANALYSIS OF MINERALS

For mineral analysis 5 g of respective dried samples were digested in concentrated HNO_3 AOAC, (2005). The digest was quantitatively transferred to a 50 ml volumetric flask and made up to volume with distilled water. A blank digest was carried out in the same way. All minerals were determined using atomic absorption spectrometry (Perkin Elmer Atomic Absorption Spectrometer A Analyst 700) against aqueous standards. The mineral concentration was expressed as mg mineral/100gm dry weight.

ANALYSIS OF ASCORBIC ACID

Sample solution equivalent to 0.2 mg ascorbic acid/ml was prepared in water containing 3% (w/v) metaphosphoric acid. It was titrated against standard 2, 6 dichlorophenol indophenol (2, 6 DCIP) solution of 0.5 mg/ml concentration until the pink color developed completely. The operation was repeated with a blank (Indian Pharmacopoeia, 1996).

ANALYSIS OF PHYTOCHEMICALS

The quantitative estimation of phytochemicals like tanins, saponin, flavonoids, alkaloids and total phenols was estimated according to Trease and Evans, (1989).

ANALYSIS OF TOTAL PHENOL CONTENT

Total polyphenols were estimated as per procedure described by Singleton *et al.*, (1999) using folin ciocalteu method, where 250 mg sample was taken in 10 ml of acetone and water (70:30 v/v) solution in a graduated test tube and heated on water bath at 70^{o}C for 10 min. The sample was brought to room temperature, centrifuged at 3500 rpm for 10 min. The supernatant (0.2 ml) was made up to 10 ml with distilled water. This solution was diluted 10 fold and sample solution (5 ml) was mixed with saturated sodium carbonate (0.5 ml) and Folin-Ciocalteaue reagent (0.2 ml) and made up to 10 ml with distilled water. The absorbance was read at 765 nm after 60 min by UV visible double beam spectrophotometer (Model Evolution 600, Thermo Electron, US).

ANALYSIS OF TOTAL CAROTENOIDS

The total carotenoid in each sample was estimated according to adapted procedure of Asharani, (2010) with some modifications. Each of the samples (5 g) was mixed with about 50 ml acetone and ground with pestle and mortar. The extract was filtered and the extraction repeated till colorless. The extracts were pooled and mixed with 50 ml petroleum ether and 400 ml distilled water in a separating funnel. The petroleum ether layer was separated and washed 2–3 times with water, dried with anhydrous sodium sulphate and made up to 100 ml with petroleum ether. The absorbance was measured at 452 nm and the total carotene content was calculated based on the molar extinction co-efficient of β-carotene.

ESTIMATION OF ACIDITY

The percent acid in the sample was determined by titrating against standard base (AOAC, 2005).

ESTIMATION OF MAJOR BIOCTIVE COMPOUNDS

ESTIMATION OF ANDROGRAPHOLIDE

Estimation of andrographolide in *Andrographis paniculata* leaves was done by spectrophotometric method (Shah *et. al*, 2007) based on the principle that condensation of g-unsaturated lactone ring of andrographolide and picric

acid in alkaline medium which results in the formation of colored complex which could be measured at 494 nm. Leaves were extracted in hot methanol, concentrated and concentrate was washed with cold toluene, washed with water and then extracted with ethyl acetate in separating funnel. Ethyl acetate was evaporated to get residue which was dissolved in methanol. Colour was developed with baljet reagent and colour was measured at 494 nm.

ESTIMATION OF BERBERINE

Estimation of berberine in stem of *Tinospora cordifolia* was done by HPLC (Srinivasan *et. al*, 2008). The stems were defatted with petroleum ether (60-80^{o}C). The marc was dried & further extracted with methanol. The concentration of berberine in methanol extract was determined using a C-18 reverse phase column with a mobile phase of acetonitrle: water (10:90 v/v) at a flow rate of 0.6 ml/min & with UV detector at 265 nm.

DETERMINATION OF RADICAL SCAVENGING ACTIVITY

The antioxidant activities of native and processed raw materials were also measured by the DPPH radical scavenging method (De Ancos, *et. al*, 2002). An aliquot (0.10 ml) of sample extract in methanol was mixed with 2 ml of methanolic 0.1 mM DPPH solution and the volume was made upto 5 ml with distilled water. The mixture was thoroughly vortex-mixed and kept in dark for 30 min. The absorbance was measured at 515 nm. The result was expressed as percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the following equation:

% inhibition of DPPH = [Abs control-Abs sample/Abs control] X 100

Where, Abs control is the absorbance of the DPPH solution without the extracts.

The radical scavenging activity was analysed in *T. cordifolia* stem, *A. paniculata* leaves and *B. diffusa* root and leaf samples.

STATISTICAL ANALYSIS

The obtained data of the proximate, mineral composition and phytochemicals of the plant parts of *T. cordifolia*, *A. paniculata* and *B. diffusa* were analyzed using one-way analysis of variance (ANOVA) and the significant differences between means were determined by post hoc Duncan's multiple range test. Differences were considered to be significant when $p<0.05$. Data were analysed using SPSS package.

RESULTS AND DISCUSSION

PROXIMATE & MINERAL CONTENT ANALYSIS

The proximate and mineral composition of selected herbs is given in Table 1 and 2. The vegetables (leaves) contain high percentage of moisture (73.02 % & 76.04 %) (Figure 1). Their contents of fat, protein & carbohydrate were low when compared to the values of 1.60 %, 25.35 % & 29.50 % reported for fairly neglected leafy vegetable *Amaranthus hybridus* (Nwaogu *et. al*, 2006) but similar to *C. nudiflora* leaf (Ujowundu, 2008). The mineral contents in each herb samples that are stem of *Tinospora cordifolia*, leaves of *Andrographis paniculata* and root & leaves of *Boerhaavia diffusa* were calcium (212, 318.62, 224, 218.24 mg/100 gm) and phosphorus (193, 250.13, 201.32, 151.45 mg/100 gm) (Figure 2) respectively. The parts have fairly adequate concentration of sodium, calcium and phosphorus, equivalent concentration of magnesium but with low concentrations of potassium and iron (Table 2) in comparison to those reported for *A. hybridicus* (Nwaogu *et. al*, 2006) and lower than *C. nudiflora* leaf (Ujowundu, 2008). The leaves were found to be richer in vitamin C, phosphorus, magnesium, calcium and manganese as compared to stem and root parts while stem and root samples of *Tinospora cordifolia* and *Boerhaavia diffusa* were found to be rich in fibre content (14.831 % & 18.3 %) respectively. Though leaves or vegetables being underutilized and neglected but they were rich in nutrients & can be utilized or consumed in diet.

Table 1 - Proximate composition of Tinospora cordifolia, Andrographis paniculata and Boerhaavia diffusa

Particulars	Tinospora cordifolia	Andrographis paniculata	Boerhaavia diffusa	
	Stem	Leaves	Root	Leaves
Moisture (%)	69.4 ±5.14^{a}	73.02±6.84^{b}	49.07±3.22^{c}	76.04±8.81^{d}
Crude fat (%)	1.03±0.03^{a}	0.98±0.02^{b}	1.69±0.03^{c}	1.16±0.02^{d}
Protein (%)	1.85±0.03^{a}	2.85±0.03^{b}	4.25±0.05^{c}	1.719±0.03^{d}
Carbohydrate (%)	9.05±0.14^{a}	12.16±0.15^{b}	22.2±3.11^{c}	17.14±2.12^{d}
Ash (%)	3.6±0.01^{a}	7.73±0.02^{b}	4.49±0.01^{c}	1.96±0.04^{d}
Crude fibre (%)	14.831±1.03^{a}	1.28±0.02^{b}	18.3±2.03^{c}	1.98±0.05^{d}

Values expressed are mean ± standard deviation of the three experiments.
Means in the same column with different letters were significantly different at $p<0.05$

Table 2 - Vitamin C and mineral composition of selected herbs

Particulars	*Tinospora cordifolia*	*Andrographis paniculata*	*Boerhaavia diffusa*	
	Stem	Leaves	Root	Leaves
Vitamin C (mg/100g)	17.01±1.78^{a}	45.01±5.23^{b}	18.65±2.48^{c}	40.00±4.32^{d}
P (mg/100g)	193±9.16^{a}	250.13±11.23^{b}	201.32±13.41^{c}	151.45±12.3^{d}
Na (mg/100g)	205±18.21^{a}	152.50±16.54^{b}	220.31±15.33^{c}	160.21±13.32^{d}
K (mg/100g)	0.97±0.02^{a}	0.93±0.03^{a}	0.99±0.02^{a}	0.86±0.01^{a}

Ca (mg/100g)	212±19.64[a]	318.62±21.02[b]	224±21.21[c]	218.24±19.44[a]
Fe (mg/100g)	0.089±0.02[a]	0.012±0.001[b]	0.098±0.03[c]	0.034±0.002[d]
Mg (mg/100g)	6.34±0.23[a]	9.68±0.06[b]	6.32±0.78[a]	8.93±0.07[c]
Mn (mg/100g)	0.29±0.01[a]	0.44±0.02[b]	0.31±0.02[c]	0.38±0.02[d]
Cu (mg/100g)	32±3.1[a]	ND[b]	65±6.12[c]	ND[d]
Zn(mg/100g)	143±7.02[a]	ND[b]	165±9.23[c]	ND[d]

Values expressed are mean ± standard deviation of the three experiments.
Means in the same column with different letters were significantly different at p< 0.05.

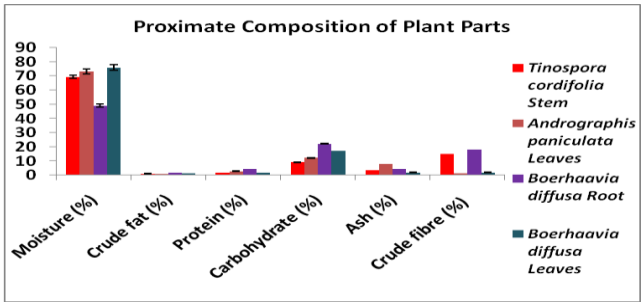

Figure 1 - Proximate composition of *Tinospora cordifolia, Andrographis paniculata and Boerhaavia diffusa*

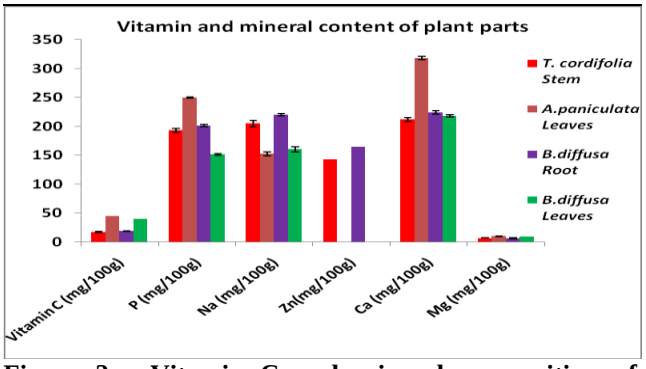

Figure 2 - Vitamin C and mineral composition of selected herbs

ESTIMATION OF MAJOR BIOACTIVE COMPOUNDS

The result of analysis of medicinally important compounds in fresh samples given in Table 3 shows that each part of the herb being analysed were rich in specific compound having medicinal properties i.e. berberine (0.03294 %) in *T. cordifolia* which was in the range of the berberine content reported by Shivkumar, (2011) i.e. from 0.002 % to 0.223 % (w/w) in different solvents (Joshi, 2013), andrographolides (1.8 %) in *A. paniculata* which was near to the results reported by Sharma *et al.*, (2012), where the andrographoloide content varied from 0.81 to 0.86 %. Results revealed that mature leaves have higher amount of andrographoloide (1.821 %) as compared to whole plant (0.831 %) (Mishra *et. al.*, 2010). Mishra, *et. al*, (2010) reported that on the variation of geographical locations and agroclimatic conditions, there was great variation in the percentage concentration of andrgrapholoide too. The *B. diffusa* leaves and roots showed 24.32 % and 18.23 percent radical scavenging activity, respectively. The Banjare, *et. al.*, (2012); Gopal, *et. al.*, (2010); Khalid, *et. al.*, (2011) and Mahesh, *et. al.*, (2012) reported strong antioxidant activity of ehanol and methanol extracts of *B. diffusa* leaves and roots and reported higher antioxidant activity of leaves than roots. The leaves of *B. diffusa* were found to be richer in percent radical scavenging activity (24.32 %) as compared to *T. cordifolia* stem (21.72 %) and *A. paniculata* leaves (23.56 %) (Figure 3).

Table 3 – Composition of bioactive compounds of selected herbs

Particulars	*Tinospora cordifolia*	*Andrographis paniculata*	*Boerhaavia diffusa*	
	Stem	Leaves	Root	Leaves
Berberine (%)	0.0329±0.002	-	-	-
Andrographoloide (%)	-	1.8±0.23	-	-
% Radical Scavenging activity	21.72±3.23[a]	23.56±4.86[b]	18.23±2.34[c]	24.32±5.01[d]

Values expressed are mean ± standard deviation of the three experiments.
Means in the same column with different letters were significantly different at p< 0.05.

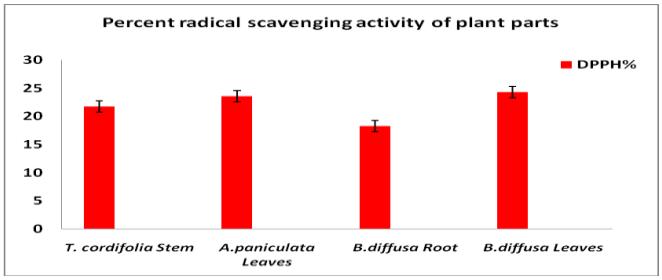

Figure 3 - Percent radical scavenging activity of selected medicinal plant parts

ESTIMATION OF PHYTOCHEMICALS

The fresh stem of *Tinospora cordifolia*, leaves of *Andrographis paniculata* and roots and leaves of *Boerhaavia diffusa* were evaluated for total phenol, alkaloid, flavonoid, saponin, carotenoid & tannin content. The present study carried out on the plant samples, revealed the presence of medicinally active constituents. The phytochemical characters of the medicinal plants investigated are summarized in Table 4. All the plant parts contain significant contents of phytochemicals. The leaves were found to be richer in phytochemicals as compared to stem and root parts. *B. diffusa* leaves were found to be

richest source of alklalloid, total phenolic content, tanins, flavonoids, percent radical scavenging activity and carotenoids among the parts analysed except saponins (Figure 3 & 4). Though leaves or vegetables being underutilized and neglected but they were rich in nutrients & can be utilized or consumed in diet. The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studied showed that the leaves were rich in phytochemicals as compared to stem and roots. They were known to show medicinal activity as well as exhibiting physiological activity. *B. diffusa* leaves were found to contain higher concentration of carotenoid, alkaloid tannins and total phenols while lower concentration of saponin and flavanoids than *A. paniculata* leaves (Dharmalingam, 2013) (Figure 4). Saponin protects the plant against microbes and fungi and is a known anti-nutritional factor, which reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physicochemical interaction, hence, it has been reported to have hypercholesterolemia effects (Price *et al.*, 1987) and thus may aid in lessening the metabolic burden that would have been placed on the liver. Phenols are said to offer resistance to diseases and pest in plants (Aliyu *et al.*, 2009). Alkaloids usually have marked physiological action on human or animals (Oomah, *et. al.*, 2003). Alkaloids are beneficial chemicals to plants with predator and parasite repelling effects. However, they inhibit certain mammalian enzymic activities such as those of phosphodiesterase, prolonging the action of cyclic AMP, affects glucagons and thyroid stimulating hormones, while some forms have been reported to be carcinogenic (Okaka, *et. al.*, 1992). It is noteworthy that at the concentration of these chemicals in edible vegetables, they are usually non-toxic. Furthermore, steaming or boiling reduces their levels in plant extracts (Piorreck *et. al.*, 1984). These vegetables also contain flavonoids, which are phenolic compounds that serve as flavoring ingredients of spices and vegetables (Enwere, *et. al.*, 1998) and referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and have antiallergic, anti-inflammatory, antimicrobial and anticancer activities (Aiyelaagbe, *et. al.*, 2009). They have been found to have anti-oxidation effects in animals. The plants studied in study can be seen as a potential source of useful phytochemical. The studies by Sarker, *et. al.*, (2012) and Khalid, *et.al*, (2012) in *B. diffusa*, Radha, *et. al.*, (2011) in *A. paniculata* and Sharma, *et.al.*, (2011) in *T. cordifolia* reported the presence of alkaloids, tannins, carbohydrates, saponins, glycosides, proteins and aminoacids, phytosterols, phenolic compounds, flavonoids and terpenoids and the present study also correlated with aforesaid studies (De MB, 2014). Presence of varieties of chemical compounds impart significant amount of biological activities of these plants.

Table 4 - Phytochemical composition of selected medicinal plant parts

Particulars	*Tinospora cordifolia* Stem	*Andrographis paniculata* Leaves	*Boerhaavia diffusa* Roots	*Boerhaavia diffusa* Leaves
Saponin (%)	1.92±0.22^a	3.23±0.11^b	1.83±0.23^c	2.74±0.03^d
Tanin (%)	0.56±0.001^a	2.613±0.02^b	0.52±0.002^c	5.2±0.06^d
Alkaloid (%)	0.25±0.001^a	0.56±0.002^b	0.15±0.001^c	0.93±0.01^d
Total Phenols (%)	5.7±0.07^a	6.31±0.11^b	5.3±0.3^c	6.5±0.20^d
Flavonoids (%)	0.81±0.02^a	1.03±0.02^b	0.79±0.03^c	0.89±0.01^d
Carotenoids (mg/100gm betacarotene)	0.98±0.01^a	1.72±0.03^b	0.76±0.04^c	5.31±0.12^d
% RSA	21.72±3.23^a	23.56±4.86^b	18.23±2.34^c	24.32±5.01^d

Values expressed are mean ± standard deviation of the three experiments.
Means in the same column with different letters were significantly different at $p< 0.05$.

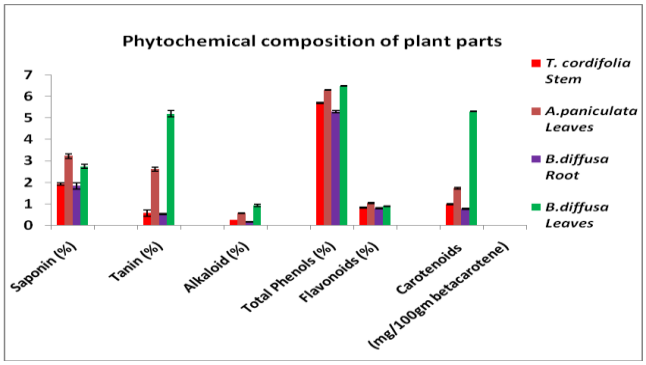

Figure 4 - Phytochemical composition of selected medicinal plant parts

ESTIMATION OF VITAMIN C

The fresh stem of *Tinospora cordifolia*, leaves of *Andrographis paniculata* and roots & leaves of *Boerhaavia diffusa* were evaluated for vitamin C. The vitamin C composition of the vegetables is given in Table 2. *A. paniculata* and *T. cordifolia* was found to contain vitamin C (45.01 and 17.01 mg/100mg), while *B. diffusa* leaves and roots found to contain vitamin C (45.00 and 40.00 mg/100g) respectively. The stem and root samples contain lesser amount of vitamin C than leaves. The plant parts contain a fair amount of vitamin C with *B. diffusa* leaves contain highest among others. The deficiency of vitamin C causes scurvy in humans and facilitates wound healing, production of collagen, formation of red blood

cells and boosts immune system. The recommended daily allowance of vitamin C is 75 mg/day for women and 90 mg/day for men (Monsen, *et. al.,* 2000). The results of the present study shows that the vegetables are of high nutritional value and may help essentially in the control of physiological oxidative stress as they have their high content of vitamin C.

CONCLUSIONS

T. cordifolia, B. diffusa and *A. paniculata* are potential sources of nutrients and some essential macro, micronutrients and phytochemical needed by man. Stem and root samples of *Tinospora cordifolia* and *Boerhaavia diffusa* were found to be rich in fibre content. The mineral contents in each herb sample were high. The leaves were found to be richer in vitamin C, minerals and phytochemicals as compared to stem and root parts. All the plant parts contain significant contents of phytochemicals. *B. diffusa* leaves were found to be richest source of alkaloids, total phenols, tannins, flavonoids, percent radical scavenging activity and carotenoids among the parts analyzed except saponins. Though leaves or vegetables being underutilized and neglected but they were rich in nutrients & can be utilized or consumed in diet. These vegetables also contain flavonoids, which are phenolic compounds that serve as flavoring ingredients of spices and vegetables. They have been found to have anti-oxidation effects in animals. Therefore, it was reasonable to determine the total primary and secondary metabolite content in the plant extract. The plants studied here can be seen as a potential source of useful drugs also. Further studies are going on these plants in order to isolate the bioactive compounds to have its application in drugs and nutraceuticals. In conclusion, the study has revealed that *T. cordifolia, B. diffusa* and *A. paniculata* are potential sources of nutrients and some essential macro, micronutrients and phytochemical needed by man. The importance of these nutrients cannot be over emphasized, to be used as vegetables. These can be incorporated in other foods as nutraceuticals for effective and proper metabolism as well as for the maintenance of good physiological state in man and animals.

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