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The effect of Climate on concentration of Saponins in *Macrotyloma uniflorum*, *Vigna unguiculata*, *Cinnamomum zeylanicum* and *Mentha piperita* using Spectrophotometer

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Abstract

Climatic variability plays an important role in secreting the secondary metabolites. Currently, most pharmaceutically important secondary metabolites are isolated from wild or cultivated plants because their chemical constituents are not economically feasible. Saponins are being promoted commercially as dietary supplements and nutraceuticals. Saponins are a class of chemical compounds found in particular abundance in various plant species. The present study was carried out to evaluate the variations in the total amount of secondary metabolites during different seasons in four important valuable medicinal plants, viz. *Macrotyloma uniflorum*, *Vigna unguiculata*, *Cinnamomum zeylanicum*, *Mentha piperita* using ultraviolet visible spectrophotometry. The plant extract was chemically treated and absorbance was measured at 544nm against blank. The concentrations of samples were calculated using standard curve. The concentration curve for saponins was determined and the correlation coefficient was calculated and was found to be 0.977 which indicates the good linearity between the concentration and the absorbance. Results revealed that maximum amount of secondary metabolites (saponins) were observed during summer, while minimum in winter season with an exception of Mu2. The concentration was almost same for Mu1 and Cz1 plant with little effect of seasonal variations and at the same place it was found to be maximum in Mu2. These plants are used in curing various diseases such as in urinary troubles. Thus present study was used as one of the parameters for standardization of medicinal plants.

Key-Words: *Macrotyloma uniflorum*, *Vigna unguiculata*, *Cinnamomum zeylanicum*, *Mentha piperita*, Saponins, Spectrophotometer, seasonal variation

Introduction

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically b y the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative.

The aglycone (glycoside-free) portions of the saponins are termed sapogenins. The number of saccharide chains attached to the sapogenin/aglycone core can vary – giving rise to another dimension of nomenclature (monodesmosidic, bidesmosidic, etc as can the length of each chain. A somewhat dated compilation has the range of saccharide chain lengths being 1–11, with the numbers 2-5 being the most frequent, and with both linear and branched chain saccharides being represented.[1]

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Dietary monosaccharides such as D-glucose and D-galactose are among the most common components of the attached chains.[2] The lipophilic aglycone can be any one of a wide variety of polycyclic organic structures originating from the serial addition of 10-carbon (C10) terpene units to compose a C30 triterpene skeleton,[3][4] often with subsequent alteration to produce a C27 steroidal skeleton. The subset of saponins that are steroidal have been termed saraponins, Aglycone derivatives can also incorporate nitrogen, so some saponins also present chemical and pharmacologic characteristics of alkaloid natural products. [6]

Horse gram *(Macrotyloma uniflorum* Lam) is a popular pulse, locally known as Gaheth belongs to the family Fabaceae that still remain an under exploited legume crop. Horse gram seeds are rich in protein and consumed in majority by poorest section of the society. [7] *Vigna unguiculata* subsp. *unguiculata* (black coloured seed coat) is a less known pulse possessing high nutritional quality [8]. The cowpea (*Vigna unguiculata* L. Walp) is considered a grain legume or

pulse which is potential agent of reducing serum lipids and glucose. *Mentha piperita* L. or peppermint with vernacular name of "nana felfeli", a plant from the Labiatae family, is traditionally used as an antiseptic, stimulant, carminative agent or it is further used as a flavoring agent in cosmetic and pharmaceutical industries throughout the world [9]. *Cinnamon* (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India. In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system [10] Environmental conditions affect the plant growth as well as the formation of secondary metabolites, as they are mostly formed in young and actively growing tissues [11]. Thus the seasonal changes have effect on the physiological parameters. Therefore, the present study was carried out to have a better understanding of plant metabolic products under seasonal influences in four important medicinal plants, *viz. Macrotyloma uniflorum*, *Vigna unguiculata*, *Cinnamomum zeylanicum* and *Mentha piperita* these plants are used in curing various diseases such as in urinary troubles and will be used as one of the parameters for standardization of medicinal plants in further studies.

Material and Methods

Collection and preparation of plant material

The plant materials were collected in two seasons i.e. summer collection in the month of July and winter collection in the month of January from the local area. The samples collected were washed, dried and authenticated and were coded as follows:

S no.	Plants studied on	Summer collection	Winter collection
1	*Macrotyloma uniflorum*	Mu1	Mu2
2	*Vigna unguiculata*	Vu1	Vu2
3	*Cinnamomum zeylanicum*	Cz1	Cz2
4	*Mentha piperita*	Mp1	Mp2

Preparation of plant extract

The whole or coarsely powdered crude drug was placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. [12]

Preparation of standard tea saponin solution

The standard tea saponin solution (0.9 mg/ml) was prepared with following protocol: 0.1000g standard tea saponin powder (containing 90% tea saponin) was weighed precisely and placed in a 100 ml volumetric flask, then dissolved with appropriate amount of 80% ethanol, and then more ethanol were added and set the volume to the mark to make it 0.9 mg/ml and shake evenly. [13] Absorbance was measured at 544nm against reagent blank.

Reagents used

Vanillin: 0.2 gm of vanillin was added to 100 ml of ethanol. [14]

Sulphuric Acid: 72 ml of sulphuric acid was mixed with 28 ml of water to obtain 72% of sulphuric acid.

Procedure

Methanolic extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60 ^{0}c for 10min, absorbance was measured at 544nm against reagent blank.[15]

Statistical analysis

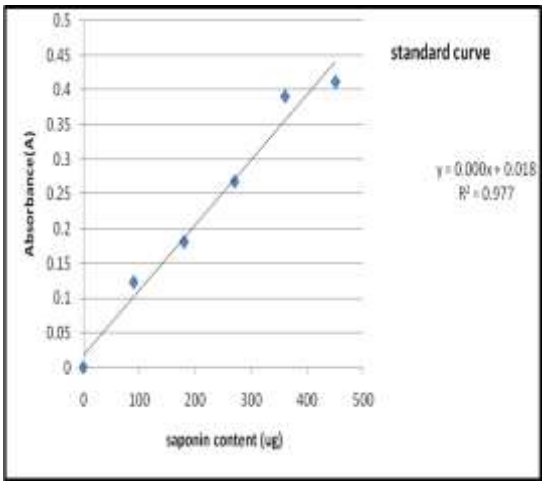
The different samples of two seasons i.e. summer collection in the month of July and winter collection in the month of January were analysed, each one in triplicate. All values are means ± standard deviation of three samples. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Differences at $P < 0.05$ were considered statistically significant.

Results and Discussion

Standard curve of Saponins

Observation for absorbance for the standard curve

S.no.	Saponins (ug)	Absorbance(A)
1.	0	0
2.	90	0.123
3.	180	0.181
4.	270	0.268
5.	360	0.391
6.	450	0.412

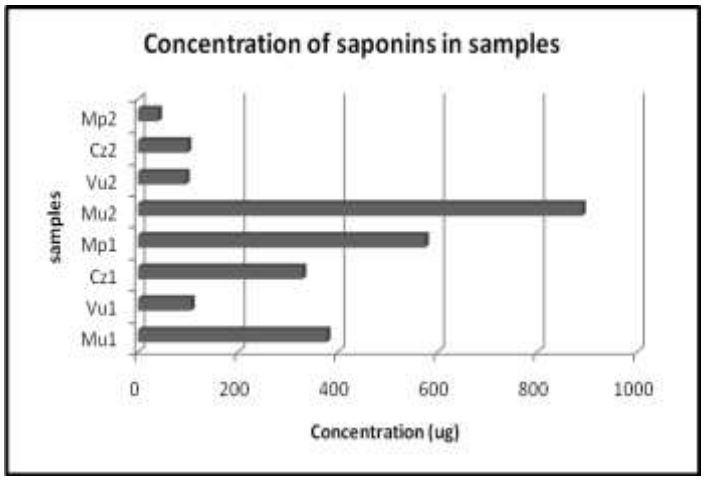


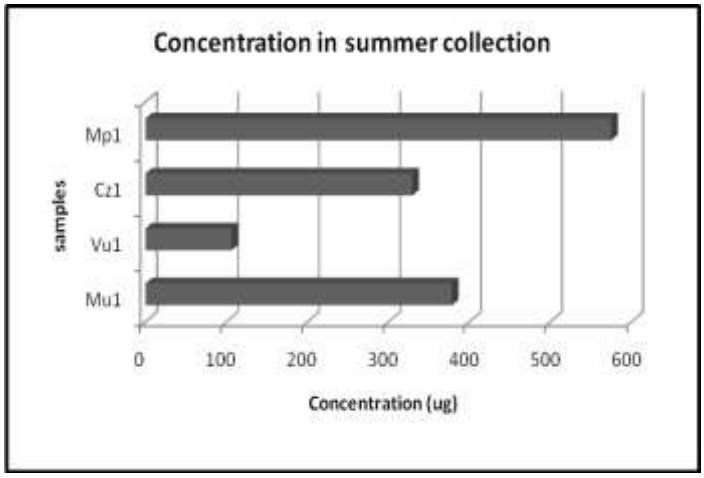
According to the standard curve, following regression equation was obtained and using the equation the concentration of sample was calculated.

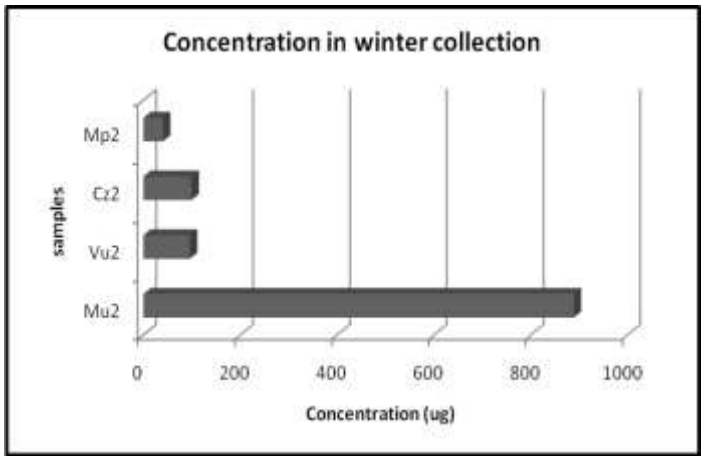
S.no.	Samples	Absorbance at 544nm	Conc.(ug)
1.	Mu1	0.359	378.44
2.	Vu1	0.114	106.22
3.	Cz1	0.315	329.55
4.	Mp1	0.536	575.11
5.	Mu2	0.819	889.55
6.	Vu2	0.105	96.22
7.	Cz2	0.108	99.55
8.	Mp2	0.055	40.66

Conclusion

The concentrations of samples were calculated using standard curve. The concentration curve for saponin was determined and the correlation coefficient was calculated and was found to be 0.977 which indicates the good linearity between the concentration and the absorbance. Results revealed that maximum amount of secondary metabolites (saponins) were observed during summer, while minimum in winter season with an exception of Mu2. The concentration was almost same for Mu1 and Cz1 plant with little effect of seasonal variations and at the same place it was found to be maximum in Mu2.







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