INTRODUCTION

Tannins and tannin-like substances are widespread in nature and are probably present in all plant materials. These are polyphenolic compounds divided into two main groups - hydrolysable and condensed. Hydrolysable tannins contain a polyhydric alcohol usually, if not always, glucose esterified with gallic acid or with hexahydroxydiphenic acid. Condensed tannins are mostly flavonoids and are probably polymers of flavon-3-ol (catechin), and these cannot be hydrolyzed to simple components. Among different chemical constituents, polyphenols (flavonoids, phenolics, condensed and hydrolysable tannins) are major bioactive compounds responsible for the prevention of chronic diseases and health care [1,2]. The therapeutic properties of herbal drugs are due to the presence of secondary metabolites which varies according to their age, seasons and maturity. They have been reported to exert anti-inflammatory, antimicrobial, antioxidant, anticarcinogenic, and body mass reducing activities. Phenolic compounds are a unique category of phytochemicals especially in terms of their vast potential healthprotective effects [3-5]. They have multiple biological effects and also act as antioxidants by preventing the oxidation of low-density lipoproteins, platelet aggregation, and damage of red blood cells. Some of these substances have antifungal, antibacterial, anticancer, and hepatoprotective effects [6-10]. 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 the majority by poorest family Fabaceae that still remain an under-exploited legume crop. Horse uniflorum and hepatoprotective effects [6-10]. Horse gram also act as antioxidants by preventing the oxidation of low-density lipoprotein. Polyphenolic compound which is proven as antioxidants and possesses a chemoprotective potential are found in leaves, flowers, fruits, and bark. The aim of the study was to estimate the amount of tannins present in these drugs using Folin-Denis method. This study was conducted to evaluate the variations in the total amount of secondary metabolites (poly-phenolic) during summer and winter seasons in the leaves of four important medicinal plants, viz., Macrotyloma uniflorum, Vigna unguiculata, Cinnamomum zeylanicum, and Mentha piperita using spectrophotometery.

METHODS

Tannin contents of plants were measured by Folin-Denis method. Tannin-like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution, the intensity of which is proportional to the amount of tannins present. The intensity is measured in a spectrophotometer at 650 nm.

RESULTS

The concentration was almost same for Mu1 and Mu2 plant with little effect of seasonal variations and at the same place it was found to be maximum in C1. The concentration curve for tannic acid was determined, and the correlation coefficient was calculated and was found to be 1 which indicates the good linearity between the concentration and the absorbance.

Conclusion

The maximum amounts of secondary metabolites (poly-phenolic) were observed during summer, while minimum in winter season with an exception of Mu2 plant. Thus, this study was used as one of the parameters for standardization of medicinal plants.

Keywords: Macrotyloma uniflorum, Vigna unguiculata, Cinnamomum zeylanicum, Mentha piperita tannins, Total phenolics, Folin-Denis method.

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Preparation of standard tannic acid solution
Tannic acid (100 g) was dissolved in 100 ml distilled water.

Preparation of working solution
About 5 ml stock solution was diluted to 100 ml with distilled water. Each ml contained 50 µg of tannic acid.

Collection and preparation of plant
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 are shown in Table 1.

Method preparation of standard curve
About 10 ml of standard solution was made up to 100 ml distilled water. 1-10 ml aliquots were taken in clear test tubes. 0.5 ml of Folin-Denis reagent and 1 ml of sodium carbonate solution was added to each tube. Each tube was made up to 10 ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 minutes and read at 650 nm against reagent blank (Fig. 1)[18,19].

Extraction of tannin
Accurately weighed 0.5 g of the powdered material was transferred to a 250 ml conical flask. Add 75 ml water. Heat the flask gently and boil for 30 minutes. Centrifuged at 2000 rpm for 20 minutes and collect the supernatant in 100 ml volumetric flask and make up the volume. Transfer 1 ml of the sample extract to a 100 ml volumetric flask containing 75 ml water. Add 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution and dilute to 100 ml with water and shaken well. Read the absorbance at 650 nm after 30 minutes.

Estimation of sample
An aliquot of the sample extract containing not more than 0.5 mg of tannic acid was used, and the percentage of tannin was determined.

RESULTS
Calculation
The tannin concentration was determined by the standard graph of tannic acid solution shown in Table 2.

| S. No. | Plants studied on | Summer collection | Winter collection |
|--------|-------------------|-------------------|-------------------|
| 1      | M. uniflorum      | Mu1               | Mu2               |
| 2      | V. unguiculata    | Vu1               | Vu2               |
| 3      | C. zeylanicum     | Cz1               | Cz2               |
| 4      | M. piperita       | Mp1               | Mp2               |

Table 1: Coding of samples in different seasons

| S. No. | Samples | Absorbance at 650 nm | Concentration (µg) |
|--------|---------|-----------------------|--------------------|
| 1      | Mu1     | 0.134                 | 1.34               |
| 2      | Vu1     | 0.088                 | 0.88               |
| 3      | Cz1     | 0.227                 | 2.27               |
| 4      | Mp1     | 0.095                 | 0.95               |
| 5      | Mu2     | 0.128                 | 1.28               |
| 6      | Vu2     | 0.049                 | 0.49               |
| 7      | Cz2     | 0.071                 | 0.71               |
| 8      | Mp2     | 0.083                 | 0.83               |

Table 2: Absorbance and concentration of total phenol in all samples

Fig. 1: Standard curve

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 analyzed, each one in triplicate. All values are means ± standard deviation of three samples. Statistical analysis was performed using a one-way analysis of variance, followed by Tukey’s multiple comparison test. Differences at p<0.05 were considered statistically significant.

DISCUSSION
The concentration curve for tannic acid was determined and the correlation coefficient was calculated and was found to be 1 which indicates the good linearity between the concentration and the absorbance. The results revealed that maximum amount of secondary metabolites (poly-phenolic) were observed during summer, while minimum in winter season. The concentration was almost same for Mu1 and Mu2 plant with little effect of seasonal variations and at the same place it was found to be maximum in Cz1. Tannins are complex...
secondary metabolites having various medicinal properties but difficult to isolate in pure form. Tannins are polyphenols, have a large influence on the nutritive value of humans and animals foodstuff. Recent interest in phenolic compounds due to their protective role, through utilization of fruits and indigenous vegetables such as apple, black caraway, carrot, cranberry, orange, and tomato against oxidative damage diseases such as arteriosclerosis, cardiovascular, coronary heart disease, aging, stroke, and cancer [20]. Many plants have been studied and reported the importance of tannins and its variation. Further study is necessary to understand the factors which may affect the production of tannins (Fig. 2).

CONCLUSION

In general, harvesting season affected the chemical composition. A significant variation in the content of total phenolics was documented. The maximum amounts of secondary metabolites (poly-phenolic) were observed during summer; while minimum in winter season with an exception of Mu2 plant. Thus present study was used as one of the parameters for standardization of medicinal plants and the information observed on seasonal variation may be useful in selecting the best season for optimal yield of the secondary metabolite of pharmaceutical importance.

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