Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2017, Volume 5, Issue 5
ISSN: 2321–3647(online)

Influence of Seasonal and Geographical Variation on Plant Metabolites of Centipeda Minima

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ABSTRACT

Traditional medicinal plants are used in treatment of number of diseases, Centipeda minima a traditional herb used in various inflammatory induced processes but still its various medicinal uses have not been identified. Centipeda minima (Asteraceae) are found in moist places. Plant being a vital component in various dosage forms therefore its quality and purity is a prime importance. Previous studies revealed that a number of active compounds have been extracted from centipede minima. But till now there is no evidence has been obtained to indicate whether the active compounds consistently present throughout different seasons and geographical conditions , present study deals with change in any major or minor variation in the plant constituents due to change in season or region. The current study indicates that secondary metabolites contents in Centipeda minima are highest in monsoon season while lowest in summer where as minor significant changes are observed in change in geographical distribution. These results indicate that the secondary metabolites quantity is highly affected by seasonal changes where as geographical distribution plays minor role. Therefore this information will give additional benefit during collection and cultivation of centipede minima.

Keywords: Centipeda minima, geographical and Seasonal Variation, secondary metabolites.

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Received 13 May 2017, Accepted 20 May 2017

Please cite this article as: Sarkar A *et al*., Influence of Seasonal and Geographical Variation on Plant Metabolites of Centipeda Minima. American Journal of Pharmacy & Health Research 2017.

INTRODUCTION

Centipeda minima is an annual plant distributed throughout high humidity geographic locations throughout China, Korea, Japan, India, Malaysia and Oceania[1] ,Six Centipeda species are reported, whole plant is harvested during summer and autumn having pharmaceutical applications [2]. It has been used in several Ayurvedic preparations as well as a number of formulations, dried herb is used to treat nasal allergies, rhinitis and sinusitis, coughs and headaches. Phytochemical investigation of plant leads to the identification of various terpenes including sesquiterpene lactones and triterpenes, flavonoids, polyphenol etc. The chief constituents namely palmitic acid, (Z,Z)-9- ,12-octadecatrienoic acid, (Z,Z,Z)-9-,12-octadecatrienoic acid,phytol, naptho[2.3-b]furan-2-(3H)-on, 1-(1,2,3,4,7,7a-hexahydro- 1,4,4,5-tetramethyl-1,3a-ethano-3aH-inden-6-yl)etanon, 1,3,5-tri-tertbutyl-benzene, (3Z)-2-methyl-3-octen-2-ol and Artemisia ketone present in Centipeda minima[3-4]. The constituents of a various medicinal plants are bound to fluctuate with seasons and geographic regions[5]. Since Centipeda minima are widely used deferent seasons, it was felt that a study on the seasonal and geographic variations will identify those changes, if any, occurring in this plant. Therefore, in the present study samples Centipeda minima were collected in April (summer), August (monsoon) and December (winter) during 2015, from Uttar Pradesh and Jammu and Kashmir. The present study deals with the study of changes in secondary metabolites like polyphenol content, flavonoids and antioxidant content due to change in seasonal and reasonal variations. Two deferent extract i.e. aqueous and hydroalcoholic are taken in experimental procedure therefore it is reported that hydroalcoholic extracts which is known to be most effective it have been compared for the plant collected during three different seasons, as well as from three different regions.

MATERIALS AND METHOD

Plant Material

Entire Centipeda minima plants were selected for the present study. The plant material was authenticated by Dr. N.K.Dubey, Department of Botany Banaras Hindu University, (U.P.). The plants were shade dried, reduced to fine powder and stored in airtight container for further use.

Preparation of Extracts

The powdered plants of Centipeda minima about 1 kg kept in soxhlet apparatus and extracted with petroleum ether, hydro alcohol (mixture of 70% ethanol and 30% distilled water) and distilled water separately, until the completion of extraction. The extract was filtered, and the

resultant extract was distilled in vacuum under reduced pressure, later dried in a desiccator's bath.

Determination of Total Phenol content

Phenol content was determined using colorimetric method 1.0 ml of the prepared each extract was oxidized using 2.5 ml of Folin- Ciocalteu reagent, and 2.0 ml of sodium carbonate solution then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. and the amount was calculated using the gallic acid calibration curve[6]. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample.

Flavonoid Content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extracts of Centipeda minima was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve [7]. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

Antioxidant Activity

Individual extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. Upper layer of solution i.e 2.5 ml was mixed with distilled water (2.5 mL) and a freshly prepared FeCl3 solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

For determination of seasonal and geographical variation of the concentration of phenolic compounds, flavonoid concentration and antioxidant activity, samples of Centipeda minima collected during three different season's i.e. from September 2014 to August 2015.Two extracts were obtained by extraction i.e. aqueous and hydro alcoholic for each seasonal sample in accordance with two different geographical conditions.

Total Phenolic Content

The aqueous and hydroalcoholic extract of Centipeda minima was used for investigation of the phenolic content in each extracts obtained from deferent seasons. Standard curve of gallic acid was calculated in distilled water for determining absorption. Phenolic content of the extracts

examined, using Folin-Ciocalteu method, total phenolic content in extracts, expressed as gallic acid equivalents. Phenolic content of hydroalcoholic and aqueous extract of Centipeda minima was found to be 92.73 and 84.22 GAE mg/g respectively. Hydroalcoholic extracts exhibited highest amount of polyphenol content compared to aqueous extracts. By analyzing the results of phenolic content in both extracts in two reasons, it was noticed that the highest concentration of phenolic compounds were obtained from plants collected during the monsoon (92.73±1.81GA/g) despite of geographical conditions. In the period during summer, the concentration of phenolic compounds was decreases 61.29±1.18 mg GA/g, increase again in samples collected during winters. Hydroalcoholic solvent system is the most effective solvent for extraction of phenolic compound from Centipeda minima. The study on geographical variations of Centipeda minima revealed that both extracts of the plants collected from Jammu and Kashmir and Uttar Pradesh, were very similar to each other only slight change in secondary metabolite reported. The absence of major variations in samples collected from the different regions indicates that the environmental conditions did not affect the chemical constituents of Centipeda minima. Minor variations, however were observed which needs to be matter of further extensive investigation.

Table 1

UP		Summer	Monsoon	winter	J & K	Summer	Monsoon	winter
	Hydro Alcoholic	72.22±1.08	91.25 ± 1.05	88.38±1.39		83.08±1.39	92.73±1.81	90.18±1.23
	Aqueous	61.29±1.18	82.61 ± 1.36	78.21±1.11		64.22±1.98	84.22±1.32	81.21±1.85

(Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations)

Flavonoid Concentration

Flavonoids are good source of medicines and used for the treatment of various diseases. The mechanism of action of the flavonoids is through scavenging or chelating processes.

The concentration of flavonoids of Centipeda minima in hydroalcoholic and aqueous extract were determined by using spectrophotometric method using aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalents. Standard curve of quercetin was calculated and plotted in distilled water for determining absorption data. The concentrations of flavonoids in hydroalcoholic and aqueous extract of Centipeda minima were reported 70.54 and 48.44 QE mg/g respectively. Hydroalcoholic extracts exhibited highest amount of flavonoids content compared to aqueous extracts. For all three seasons highest concentrations of flavonoids were obtained in hydroalcoholic extracts. The highest concentration of flavonoids

(70.54±1.77mg RU/g) was recorded during monsoon season, reported decline in the concentration during summer season extracts i.e 42.90±1.75and 43.66±1.35mg RU/g, again at the start of winter, flavonoid concentrations increases regularly.

Table 2

UP		Summer	Monsoon	winter	HP	Summer	Monsoon	winter
	Hydro Alcoholic	61.34±1.35	69.21 ± 0.52	66.41±0.64		65.74±1.75	70.54±1.77	68.01±0.04
	Aqueous	42.90±1.75	47.38 ± 0.73	45.37±1.58		43.66±1.35	48.44±1.78	46.17±1.89

(Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations)

Antioxidant Activity

The findings of polyphenol and flavonol content of hydroalcoholic and aqueous extract of Centipeda minima supports the study of DPPH scavenging capacity of extracts. The hydroalcoholic and aqueous extract of Centipeda minima were subjected to in vitro antioxidant studies to determine and compare the antioxidant activities of both extracts. DPPH is stable nitrogen centered free radical that can adopt an electron or hydrogen radical to become a stable diamagnetic molecule[7]. DPPH radicals act with appropriate reducing agents, and then depriving colour stoichometrically with the number of electrons depleted which is measured spectrophotometricallty at 517 nm [8]. Hydroalcoholic and aqueous extracts of Centipeda minima strongly scavenged DPPH radical with the IC50 being 59.02 and 97.42 µg/ml, respectively. The hydroalcoholic extracts exhibited highest scavenging property compared to aqueous extracts.

Table 3

UP		Summer	Monsoon	winter	J & K	Summer	Monsoon	winter
	Hydro Alcoholic	59.02 ± 0.13	95.66 ± 1.12	60.73 ± 0.05		61.37 ± 0.72	97.42 ± 0.58	61.37 ± 0.72
	Aqueous	29.32 ± 0.43	85.16 ± 1.12	59.73 ± 0.95		32.32 ± 0.33	88.16 ± 1.12	72.73 ± 0.64

(Values are mean ± SEM of six determinations)

DISCUSION

Plant metabolites produces large number of specialized compound, required for the survival of plant, divided into two classes i.e. primary and secondary metabolites. Primary metabolite governs all physiological processes where as secondary metabolites like terpenoids, plyphenols, flavonoids regulates growth and development of the plant[9].change in the amount of secondary metabolites depends on biotic and abiotic factors [10]. Present study deals with the estimation of secondary metabolites with seasonal and geographical changes, Secondary metabolites like

polyphenols found in large quantity in plants therefore we have to estimate amount of polyphenols in different extracts obtained from collection during winter, summer and Monsoon, results shows that hydroalcoholic extracts having greater concentration while comparison with aqueous extracts, therefore it is better to go forward with hydroalcoholic extracts. Similar results are obtained in falvonoids and antioxidant activity in hydroalcoholic and aqueous extraction procedure. Results of the study shows that hydroalcoholic extracts are more prominent for extraction of centipede minima. Samples of centipede minima collected in three deferent seasons having shown remarkable change in metabolite contents. This indicates that climate does affect the chemical spectrum for the plant widely, study on different geographical extracts of Centipeda minima revealed that the plants collected from Jammu & Kashmir and Uttar Pradesh were almost similar to each other irrespective of certain minor changes[11]. Our study sets at rest the speculations on the probable variations in chemical constitutions induced by the seasons and environment. Not every plant can be cultivated everywhere[12], some plant is able to grow in deferent regions, the phytochemicals of the plant growing in two different regions are found to be slightly different. Thus it indicates that plants produce various secondary metabolites as a result of their interaction with the environment. Therefore change of season or region does affect the synthesis of phytochemicals of Centipeda minima. This is a very positive result as far as standardization of the plant material is concerned. Similar analysis need to be carried out for all other plants to certify its consistency before they are used to prepare formulations.

CONCLUSION

The current study indicates that there is a difference in phenolic content, flavonoid content and antioxidant activity calculated in deferent extracts during the three deferent collections time i.e. summer, winter and Monsoon, and their compositions vary significantly. It is noticed that the highest concentration of secondary metabolites are obtained by using hydroalcoholic solvent. Therefore, present study can be useful to get a higher concentration of these substances since this species has a long history of commercial use. Change of season does affect drastically whereas change in region shows marginal variation of plant constituents, therefore this study deals that plant samples of Centipeda minima must be collected during Monsoon Season for better phytochemical findings rather than collection in winter or summer, findings of the results also indicates that Centipeda minima can be considered to show enough consistency in their secondary metabolite content irrespective of the place of collection.

ACKNOWLEDGEMENT

We thank to the Institute of Pharmacy, HCPG College, Varanasi, Uttar Pradesh, India for providing research facilities to carry out research work.

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