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Temporal variation in the quantitative estimation of total phenolic and flavonoid contents of two species of *Calotropis*

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ABSTRACT

Phenols and flavonoids present in medicinal plants are considered to be among the most important bioactive components. *Calotropis procera* and *Calotropis gigantea* are much alike medicinal plants with a wide range of bioactivity. The present study was aimed to evaluate the effect of time on total phenolic content (TPC) and total flavonoid content (TFC) in the methanolic extracts of leaf and flower tissues of *C. procera* and *C. gigantea*. TPC and TFC were estimated by spectrophotometric method using gallic acid and quercetin as respective standards. The samples were collected in morning, afternoon and evening session's. Significant variation in TFC and TPC levels was observed in between the selected species. *C. procera* presented highest TPC in leaves harvested in afternoon (20.10 mg/gm) and highest TFC in flowers collected in evening (36.755 mg/gm). In conclusion, the present investigation demonstrates that there is significant effect of harvesting time of different tissues of *C. procera* and *C. gigantea* on their TPC and TFC contents.

KEY WORDS: *C. GIGANTEA, C. PROCERA, GALLIC ACID, METHANOLIC EXTRACT, QUERCETIN, TFC, AND TPC*

INTRODUCTION

Secondary metabolites are the natural phytochemicals which owe medicinal importance to the plants they belong (Justin *et al.*, 2014). Plant secondary metabolites are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction (Marinova *et al.*, 2005). They act in defense purposes to protect a plant from any possible harm in the ecological environment (Stamp *et al.*, 2003). Phenolic compounds are aromatic secondary plant metabolites broadly distributed throughout the plant kingdom (Mamta *et al.*, 2012). They confer unique taste and flavor to the plant and plant derived products (Omas-Barberan and Espin, 2001). Phenolic compounds are one of largest group of secondary metabolites synthesized by plants. They offer great deal of health ben-

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efits to the mankind which include their antioxidant, anti-inflammatory, anti-carcinogenic and other biological properties like reduction in blood cholesterol and lipid levels, delaying the development of chronic diseases such as cancer and Alzheimer's disease (Park *et al*., (2001), Ali *et al*., (2011) and Bodeker, (2000). Flavonoids are a class of polyphenols which are water soluble pigments found in the vacuoles of plant cells (Justin *et al*., 2014). The role of flavonoids in flowers is to act as colouring agents for plant pollinators (Harborne, 1976) and in leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from fungal pathogens and UV-radiation (Harborne, 1993). They are anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral. (Guardia *et al*., 2001).

Calotropis gigantea L. (family Asclepiadaceae), commonly known as giant weed or milkweed, is a usual wasteland plant found along degraded roadside and overgrazed pastures (Caius 1986 and Sharma, 1954). Calotropis is drought impermeable and salt tolerant weed growing upto an altitude of 900 m asl throughout the country (Mueen *et al*., 2005). It prefers distressed sandy soils with mean annual rainfall: 300-400 mm. It has clusters of waxy flowers that are either white or lavender in colour. *C. gigantea* is a perennial herb with wide pharmacological significance in traditional and Unani systems of medicine. The flowers and bark with milky latex are reportedly known for various biological activities including analgesic, antimicrobial, antioxidant, anti-pyretic, insecticidal, cytotoxic and hepatoprotective activity (Sarkar and Chakravarty, 2014). A wide range of chemical compounds including cardiac glycosides, flavonoids, terpenoids, alkaloids and resins have been isolated from this plant (Singh *et al*. 2014). Recent studies have shown that, *C. asiatica* accumulates major phytoconstituents in the months of summer season (Alqahtani *et al*., 2015). Moreover, studies on *Ribes nigrum* and '*Zonouz*' peel have shown that harvesting time and leaf position have a profound effect on their phenolic contents, (Hallman *et al*., 2013 and Michael *et al*., 2015).

Calotropis procera L. (family Asclepiadaceae), commonly known as "Sodom apple" is well known for its high medicinal properties. *C. procera* is drought-resistant, salt-tolerant, animophilous or antomophilous plant. The plant grows along degraded road sides, lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation, especially sandy soils in areas of low rainfall (Sharma *et al*., 2011). The leaves are useful in the treatment of paralysis, arthralegia, swelling and intermittent fevers. Methanolic and aqueous extracts of leaves of *C. procera* have been reported to have the potential of antioxidant and antibacterial activity (Patel *et al*., 2012). The present investigation was aimed to study the effet of collection of two different tissues from two Calotropis species on their TPC and TFC contents. Quantitative measurement shows higher phenolic and flavonoid content in leaf and flower tissues of *C. procera* as compared to that in *C. gigantea*.

MATERIALS AND METHODS

Gallic acid and Quercetin (MERK®), Methanol (MERK®), Folin-ciocalteu Reagent (MERK®) (1:10 in deionised water), Sodium carbonate solution (7.5% w/v) (MERK®), Sodium nitrate (MERK®), Alcl3 and NaOH (MERK®). Gallic acid and Quercetin (3 mg) were accurately weighed into a 10 ml volumetric flask, dissolved in small amount of distilled water and the solution was made up to 3 ml with the same solvent to make the final stock of 1 mg/ml.

The aerial parts (leaves and flowers) of the plants were collected in the months of March-April, 2014 from MPCST 23° 15′ 35. 760″ N and 77° 24′ 45. 414″ E and P&T Colony 23° 13′ 25. 582″ N and77° 23′ 28. 294″ E Bhopal, India. The collected plant samples were identified by Dr Zia ul Hasan (Prof and Head, Faculty of Botany, Saifia Science College, Bhopal, India). The specimen samples of *C. procera* and *C. gigantea* were deposited in the departmental herbarium with respective voucher number 481/Bot/Saifia/2014 and 482/Bot/Saifia/2014.

The plant materials were collected at different intervals of time: (Dawn 6:30 am), (Noon 12:30 am) and (Dusk 6:30 pm) by hand plucking. The samples were washed thoroughly with the distilled water to remove the dirt and other contaminations followed by careful drying under shade at room temperature in order to prevent fungal infection and decomposition of active compounds. The dried leaves and flowers were powdered using a grinder and stored in airtight packing polythenes until required for use.

Extraction was carried out by employing the maceration method. Equal quantities (100 g) of powdered tissues of leaf and flowers were taken and put in 500 ml plastic container. These samples were macerated with 500 ml of 75% Methanol. Each was allowed to stand for two weeks with constant shaking at regular intervals under room temperature. After maceration samples were fine filtered through muslin cloth and then by Wattman filter paper and the solvents were evaporated to obtain the methanolic extracts of the leaves and flowers. These served as the stock solutions which were stored in a dry and cool place until needed for analysis.

The amount of total phenolics in the extracts was determined with the Folin- Ciocalteu reagent (Ainsworth and Gillespie, 2007). Gallic acid was used as a standard and the total phenolics were expressed as mg/g Gallic acid Equivalents (GAE). For this purpose, the calibration curve of Gallic acid. 1 ml of a standard solution of concentration 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of

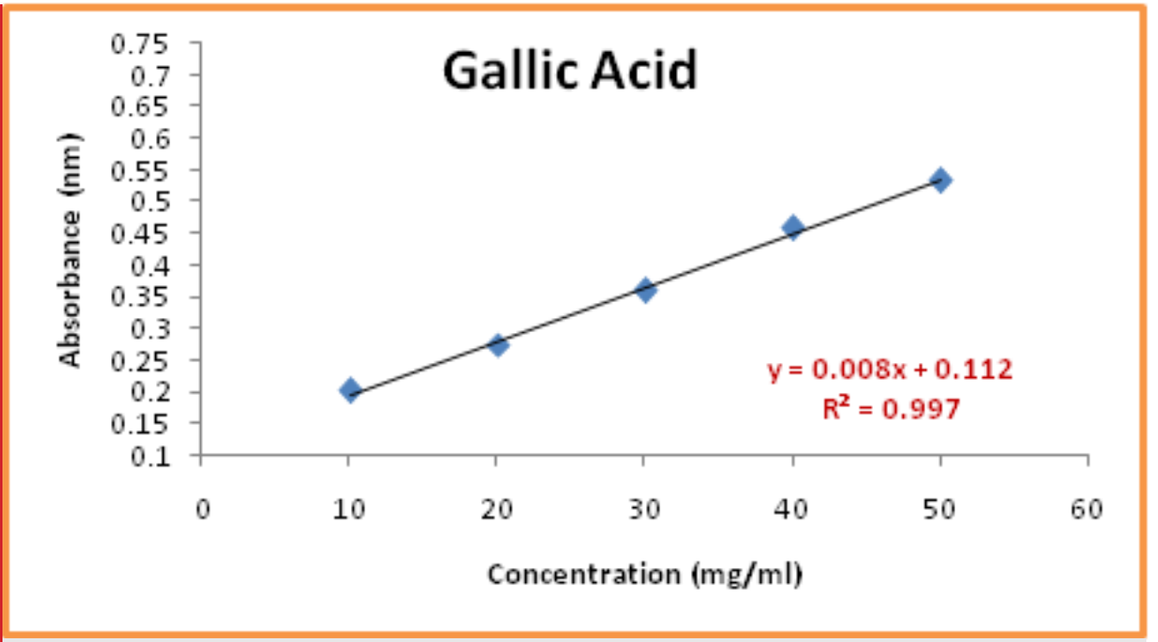

FIGURE 1: **Standard Curve of Gallic acid for Total Phenolic content**. Line of regression from gallic acid was used for estimation of unknown phenolic content. From the standard curve of gallic acid, line of regression was found to be: Y= +0.0085x + 0.1121 and R2= 0.997

Gallic acid was prepared in methanol. A concentration of 0.1 or 1 mg/ml of plant extract was also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute Folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The test tubes were allowed to stand for 30 minutes at room temperature and the absorbance was read at 760 nm spectrometrically.

TOTAL FLAVONOID CONTENT

A double beam UV/Visible spectrophotometric method was used to estimate the flavonoid content (Zhishen *et al*., 999). A standard solution of quercetin was added to 75 µl of $NaNO_2$ solution and mixed for 6 min before adding 0.15 ml of $Alcl_3$ (100 g/l). After 6 min, 0.5 ml of NaOH was added. The final volume was adjusted to 5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was taken at 510 nm against water as blank. Total flavonoid content was expressed as mg quercetin/g dry weight of methanolic extract.

RESULTS AND DISCUSSION

Calotropis sp (Ait.) R. Br., a wild growing plant of family Asclepiadaceae, is well known for its medicinal properties. Different parts of this plant have been reported to exhibit anti-inflammatory, analgesic, and antioxidant properties (Dwivedi *et al*., 2010). Plant secondary metabolites are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction. Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, hypoglycemic and anti-carcinogenic (Borneo and katalinic, 2011). Phenols are a class of secondary metabolites derived from the Shikimic acid pathway. They are believed to function in plant defense mechanisms against insect herbivores and fungi (Nikam *et al*., 2012).

Flavonoids as secondary metabolites are believed to act in defense related sinalling pathways in the plans against an array of biotic and abiotic stress related conditions (Marinova *et al*., 2005) . Effect of time of collection (seasonal variation) was evident in the content of TFC and TPC constituents of the two species of Calotropis. The amount of the total phenol was estimated with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent (Fig. 1).

The highest phenolic content (TPC) was found in the methanolic extract (20.10 mg/gm) of C. the Quercetin reagent. Quercetin was used as a standard compound

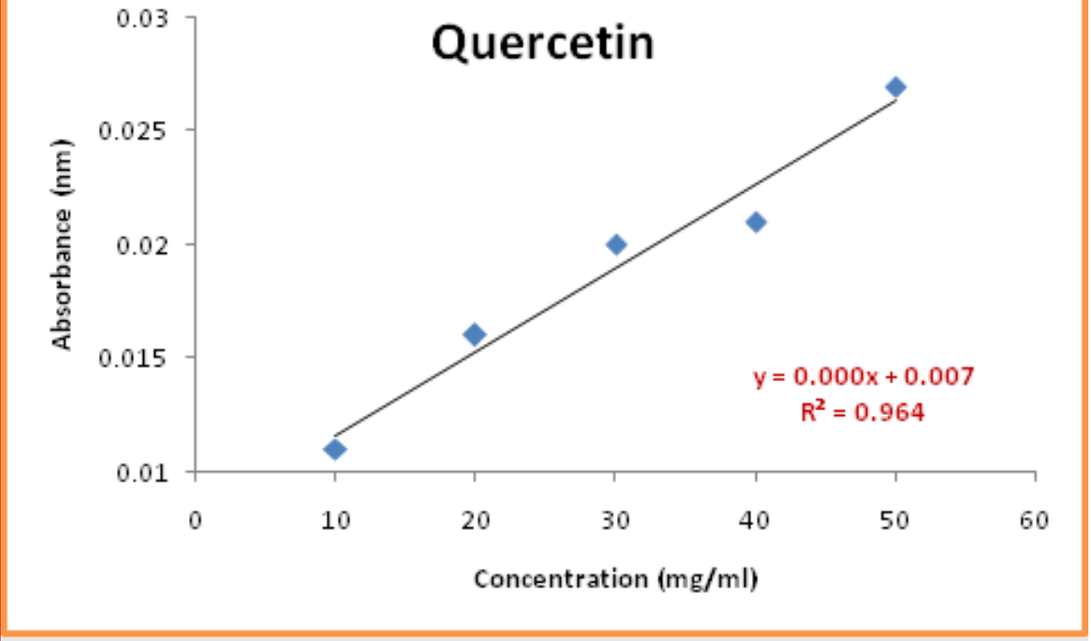

FIGURE 2: **Standard Curve of Quercetin for Total Flavonoid content.** Line of regression from qurcetin was used for estimation of unknown flavonoid content. From the standard curve of qurcetin, line of regression was found to be: Y= 0.004x + 0.0079 and R2= 0.9641.

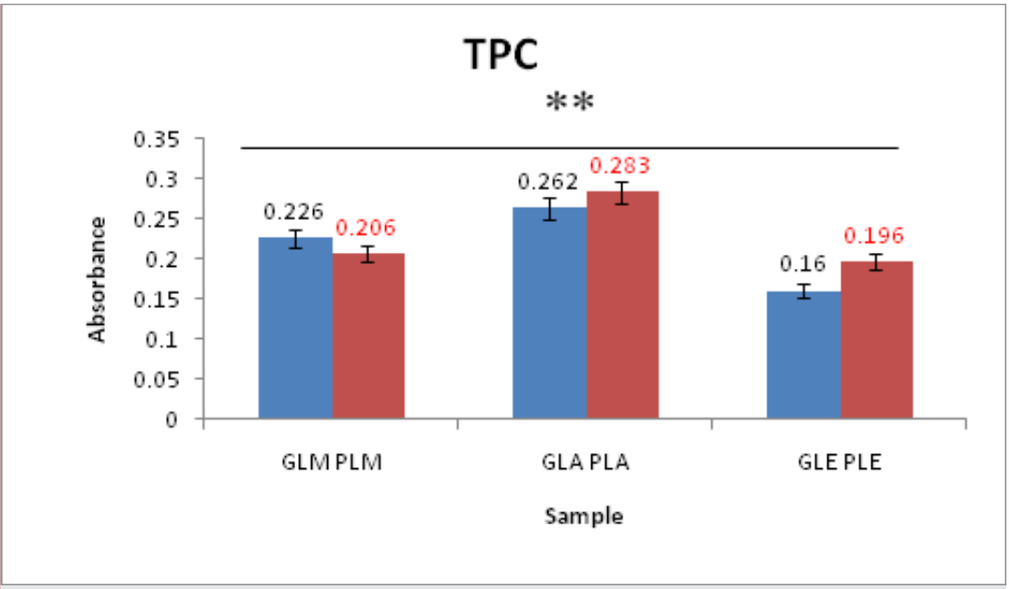

FIGURE 3: **TPC Of *Calotropis procera* leaves and TPC of *Calotropis gigantea* leaves.** Signs: GLM (Gigantea leaves Morning), GLA (Gigantea leaves Afternoon), GLE (Gigantea leaves Evening) PLM (Procera leaves Morning), PLA (Procera leaves Afternoon), PLE (Procera leaves Evening). Data are mean ± S.D. of three similar experiments. *P < 0.05; **P < 0.01.

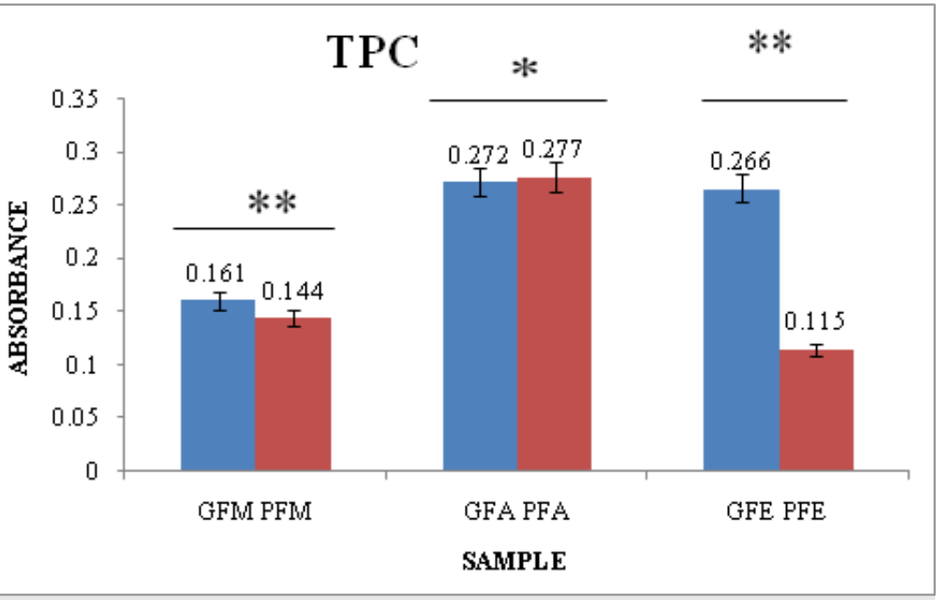

FIGURE 4: TPC Of *Calotropis procera* flowers and TPC of *Calotropis gigantea* flowers: Signs: GFM (Gigantea Flowers Morning), GFA (Gigantea Flowers Afternoon), GFE (Gigantea Flowers Evening) PFM (Procera Flowers Morning), PFA (Procera Flowers Afternoon), PFE (Procera Flowers Evening). Data are mean ± S.D. of three similar experiments. *P < 0.05; **P < 0.01.

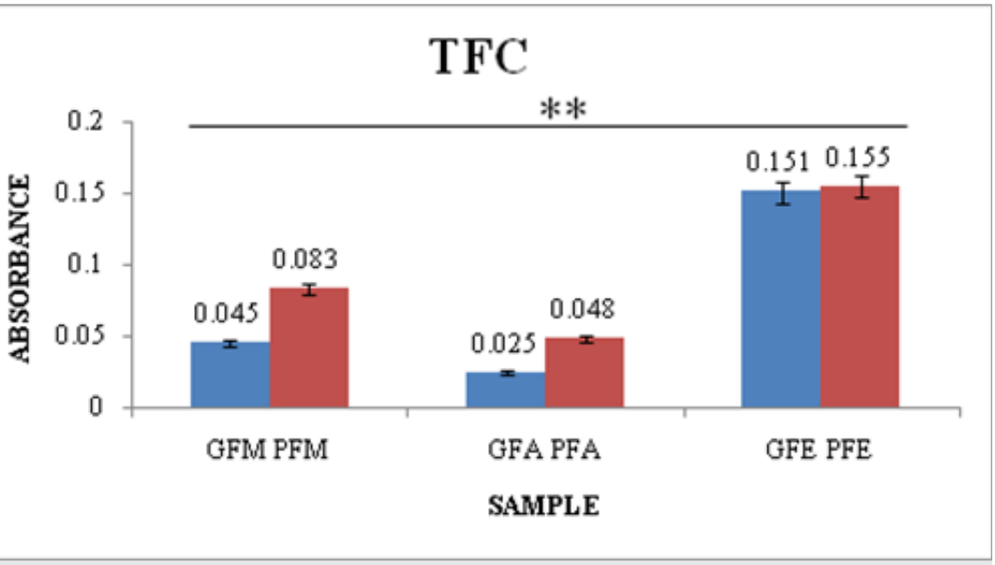

FIGURE 5: TFC Of *Calotropis procera* flowers and TFC of *Calotropis gigantea* flowers: Signs: GFM (Gigantea Flowers Morning), GFA (Gigantea Flowers Afternoon), GFE (Gigantea Flowers Evening) PFM (Procera Flowers Morning), PFA (Procera Flowers Afternoon), PFE (Procera Flowers Evening). Data are mean ± S.D. of three similar experiments. **P < 0.01.

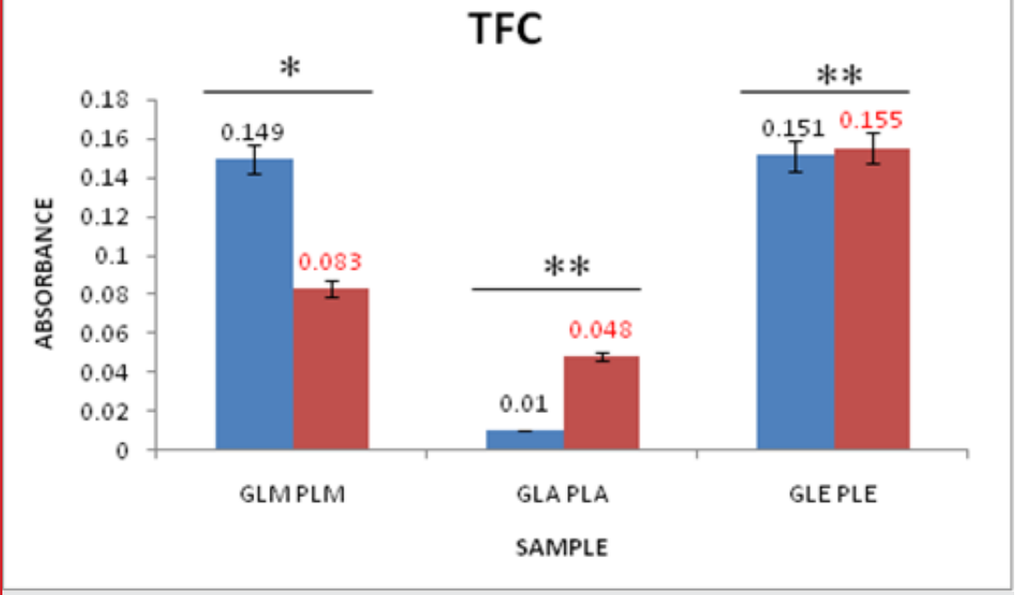

FIGURE 6: **TFC Of *Calotropis procera* leaves and TFC of *Calotropis gigantea* leaves:** signs: GLM (Gigantea Leaves Morning), GLA (Gigantea Leaves Afternoon), GLE (Gigantea Leaves Evening) PLM (Procera Leaves Morning), PLA (Procera Leaves Afternoon), PLE (Procera leaves Evening). Data are mean ± S.D. of three similar experiments. *P < 0.05; **P < 0.01.

and the Total falvanoids were expressed as mg/g Quercetin equivalent (Fig. 2). The highest falvanoid content (TFC) was found in the methanolic extract (36.755 mg/gm) of *C. procera* flowers harvested at evening (Fig. 5 and Fig. 6). Our observations were in agreement with earlier such studies on *Tecomella sp.* wherein TPC and TFC where found to be higher in summer season than in winter or monsoon seasons (Patel and Patel, 2014).

Similar studies have been reported on *C. asiatica* wherein it was shown to accumulate major phytoconstituents in the months of summer season (Alqahtani *et al.*, 2015). Moreover, studies on *Ribes nigrum* and '*Zonouz*' peel have shown that harvesting time and leaf position have a profound effect on their phenolic contents and (Michael *et al.*, 2015 and Hallman *et al.*, 2013). The place of cultivation and the harvesting period affects the phenolic compounds in fruit tissues of two *C. asiatica* cultivars (Puttarak and Panchayupakaranant, 2012).

Here we have observed that time of collection has profound prospect in ascertaining the drug quality and further selection of elite chemovariant among the two species of *Calotropis*. However, the present study needs further investigation as to how the chemical composition of different tissues is affected by period of harvesting.

CONCLUSION

The present study revealed that time of collection has special effects on total phenolic content (TPC) and total flavonoid content (TFC) of *C. gigantea* and *C. procera*. Quantitative measurement shows higher phenol and flavonoid content in leaf and flower tissues of *Calotropis procera* in afternoon and evening sessions compared to *Calotropis gigantea*. Such studies have a prospect in deciding on proper timing of collection visavis selection of elite chemotype and further exploration for drug design and development.

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