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Effect of seasonal and lunar cycle variability on phytoconstituents of *Calotropis Procera* (Ait.) R. Br. Aerial parts

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Abstract
Calotropis procera is wild, evergreen plant belongs to *Asclepiadaceae* family and used in an Indian traditional system of medicine since ancient times. *C. procera* employed the drug bestowed with R. *asaviryadi* (therapeutic principle and potency*)* quality must be collected to achieve good therapeutic results. Therefore, advocate the collection practice and seasonality that may affect qualitatively and quantitatively to the drug. This is because moonlight is stronger than sunlight. The study aims at seasonal and lunar based aerial parts collection, extraction and quantitative phytochemical analysis. This study found a significant shift in the production of bioactive metabolites during the lunar cycle (full moon and new moon). Results revealed maximum carbohydrate and protein content were found in leaves and floral at PSFM respectively. Flavanoid (76.89±1.02 mg g^{-1}) and phenolics (84.41±1.09 mg g^{-1}) values were higher in leaves at PWFM and PWNM while tannin and cardiac glycoside (80.67±1.44 mg g^{-1}) attained maximum content at PRFM and PSFM in buds respectively. The study of lunar and seasonal based variations of *C. procera* phytoconstituents emphasize that effective and concentrated drugs can be extracted during the full moon to treat various diseases globally including local people with a minimum exploitation rate.

Keywords: *C. procera*, seasonal variation, optimum production, cardiac glycoside, moon light, lunar cycle

1. Introduction
Calotropis procera, a shrub belonging to Asclepediaceae (milk bearing) family commonly known as RAKTA ARKA or Sodom apple. *C. procera* is a valuable wild medicinal plant used in therapeutically and agriculture (an herbicide) from classic to a modern era and it has salient features like drought resistance, branched roots, and evergreen plant with latex. It is one of the richest sources of bioactive constituents which are synthesized by plants, required for their survival and protection. Therapeutically rich secondary metabolites of aerial parts of *Calotropis procera* like flavanoids, phenolics, cardiac glycoside, terpenes, tannins, etc are distributed in all parts of the plant (Mossa *et al*., 1991, Kaur *et al*., 2021) [1, 2].
Different parts contain various chemical diversity of secondary metabolites having immense therapeutic potential in the Indian traditional system (Kadiyala *et al*., 2013) [3] But the total relative distribution may vary with ecological factors (Moustafa *et al*., 2010) [4]. There are several ethanomedicinal drugs reported from the parts of this plant. The phytochemical composition is mostly influenced by many climatic (abiotic) components like altitude, seasons, different types of soil, day length, night length, habitat, and collection time, etc. Because variation in metabolite production does not depend only on genetic factors, environmental conditions, morphological characters, and the methods of collection also play a vital role (Al-Rowaily *et al*., 2020, Sivaci *et al*., 2014, Tavhare *et al*., 2015) [5, 6, 7]. *C. procera* is traditionally used to treat cough, eczema, rheumatism, cold, elephantiasis, etc. flowers are considered as digestive, stomachic, tonic and useful in cough, asthma catarrh and loss of appetite. Leaves are used as eliminate joint pain and minimize the swelling and tender leaves are used to cure migraine. It is reported that the aerial plant parts (floral, leaves, apical buds) exhibited a variety of pharmacological activities like Floral exhibited anti-asthmatic and anticancer activity, leaves show antidiabetic, antipyretic, anti-candida activity, cytotoxic activity while stem bark exhibited anticonvulsant activity and ethanolic extract of aerial parts exhibited antipyretic activity ((Kadiyala *et al*., 2013, Pattnaik *et al*., 2017, Tavhare *et al*., 2016) [3, 8, 9].

In developing countries, drastic depletion of plants, human disturbance in plant resources, and soil erosion are increasing day by day. Hence, there is indeed a need to protect plant resources by applying proper harvesting and collection methods, which in turn ultimately affects the therapeutic efficiency as well as the optimum concentration of the drug [7, 9]. A number of studies reported on seasonal variation of medicinal plants, but the current study was focused on seasons with lunar cycle variation. 60% of tidal force is due to the moon and 30% is due to the sun and hence, moon-light are stronger than sunlight which penetrates in soil and transforms the plant life (Barlow *et al.*, 2012) [10]. For that reason in modern era, researchers are drawn towards the studies on the significant impact of full moon, new moon and seasonal variation on active phytoconstituents. Moreover, the syntheses of active metabolites are drastically altered by forces of the gravitational attraction in the lunar cycle during new moon (*Amavasya),* full moon *(Purnima*), and water content of the soil is affected as tides. However, the phytochemical profiling of aerial parts of *C.procera* remains to be unexplored and to the best of my knowledge, this may be the first report based on collection in seasonal and lunar cycle till date. Thus, this study was designed to focus on Effects of seasonal and lunar cycle variability on phytoconstituents of *Calotropis Procera* (Ait.) R. Br. aerial parts (Leaves, Florals, and Apical buds).

The main objective was to get a high-quality drug, considering the lunar cycle and seasonal variation. Thus the effect of the full moon, new moon, and season could increase the optimum quantity of active metabolites of *C. procera* for a maximum and active therapeutic drug.

2. Materials and methods

2.1 Collection and Authentication of plant:

C. procera aerial plant parts (leaves, flower, and apical buds) were collected between 9:00 am - 11:00 am to keep the "time" variable constant; during the seasons: Winter (November-December), Summer (April-May), and Monsoon (July–August) of the year 2016 considering as the peak months for three seasons during the day after New Moon(NM) and Full Moon (FM) from Uka Tarsadia University, Maliba Campus, Tarsadi (was Latitude - 21˚12", Longitude - 73˚11" 30 m above the sea level). The collected plant was identified at Navsari Agriculture University, Navsari, India. The voucher specimen for *C. procera* (UTU/CGBIBT/17^{-1}8/03) was deposited at CGBIBT, UTU, India.

2.2 Preparation of extracts

C. procera plant parts were washed thoroughly with water and shade dry at room temperature (RT) (37˚C) for 10 days. After drying, the samples were ground to a coarse powder and store it for further analysis. Powdered samples (5 gm of each) were extracted with 50 ml of sterile water and macerate for 2 hrs at RT with continuous shaking. Filtered the resulting extracts with what-man filter paper no.1 (0.11µm) and concentrated to dryness by evaporation using a rotary evaporator. The yielded extracts were stored for further analysis.

2.3 Quantitative phytochemical evaluation of *C. procera*

Quantitative analysis was carried out to check the availability of various phytoconstituents in a specific quantity of the plant parts extracted during all the seasonal and lunar cycles.

2.4 Determination of Total Carbohydrate Content (TTC)

The carbohydrate content of *C. procera* extracts was estimated by modifying the anthrone method (Morsy *et al.*, 2012) [11].1 ml of plant part extracts added in 4 ml of acidic anthrone reagent (SD fine, Pvt. Ltd.). Boiled in a boiling water bath for 8 min. Cooled rapidly and read the green to dark green color at 630 nm on a spectrophotometer (Shimadzu, UV-1800). The amount of total carbohydrate expressed in the mg g^{-1} of glucose (GLU).

2.5 Determination of Total Protein Content (TPRC)

Total protein content for *C. procera* extracts was estimated by Lowry's method, based on under alkaline condition the reactivity of the peptide nitrogen(s) with the cu^{++} ion and subsequent reduction of the Folin-Ciocalteu(FC) phosphomolybdic phosphotungstic acid to hetero poly molybdenum blue [11]. 100µl of extract mixed with 500µl of alkaline copper sulphate reagent and incubated for 10 min at RT. 50µL of FC reagent was added and incubated for 30 min at room temperature in dark. The blue color developed was read at 660 nm on a spectrophotometer. Protein content was expressed in mg g^{-1} of the dry mass of Bovine Serum Albumin (BSA).

2.6 Determination of Total Phenolic Content (TPC)

The total phenolic content for *C. procera* extracts was determined using the modified Folin Ciocalteu method (Kumar *et al.*, 2013) [12]. It works by measuring the amount of substance needed to inhibit the oxidation of the reagent. A Calibration curve was prepared with gallic acid. 100 µl extract mixed with 500 µl FC reagent (1:1 diluted) including the blank after 5 minutes added 2.5 ml of aqueous Na_2CO_3 (20%), vortexed well and incubated in dark for 40 minutes. The presence of phenolics was read at 725 nm. All determinations were carried out in triplicate. Total phenolic values were expressed as Gallic acid equivalents per gm material (mg g^{-1} dry mass).

2.7 Determination of Total Flavanoid Content (TFC):

The total flavanoid content for *C. procera* extracts was determined by the aluminum chloride method in which it forms acid - stable complexes with the C- 4 keto group and either the C-3-C-5 hydroxyl group of flavones and flavonols was adapted from Lillan barros with some modification (Barros *et al.*, 2008) [13]. 100 µl of *C. procera* parts extract added in 100 µl 5% $NaNO_2$, after 6 min of incubation add 100 µl 10% $Alcl_3$. Incubate it for 6 minutes, and then add 500 µl of 4% NaOH followed by 2500 µl D/W. The presence of flavanoid was read at 510 nm. Total flavanoid value was expressed as Rutin equivalents (mg g^{-1} dry mass). All fractions were run in triplicate.

2.8 Determination of Total Tannin Content (TTC)

Total Tannin Content for *C. procera* extracts was determined by the Phenol-Denis method (Khadabadi *et al.*, 2013) [14]. 1 ml of the plant extracts and standard was mixed with 0.5 ml Folin's phenol reagent (FCR) followed by 5 ml of 35% sodium carbonate. The mixture was incubated for 5 min at *RT*. The blue color produced was read at 640 nm standard using UV/visible spectrophotometer. The tannin content was calculated by the calibration curve of Gallic acid and the results were expressed as Gallic acid equivalent (mg g^{-1}).

2.9 Determination of Total Cardiac Glycoside Content (TCGC): Total Cardiac Glycoside content for *C. procera* extracts was determined by DNBA (3, 5-dinitrobenzoic acid) method (Khadabadi, 2013, Gupta *et al.*, 2008) [14, 15]. 1 ml of

DNBA was added in 1 ml of all plant parts extract and tubes were mixed well with vortex, 1 N of 100 µl sodium hydroxide was added in all test tube and mixed well. The resulting color was read at 565 nm to give optical densities proportional to test concentrations. TCGC value was expressed as Digoxin equivalent (mg g^{-1} dry weight).

2.10 Statistical analysis
The quantitative phytochemicals results obtained were analyzed using Principle Component Analysis (PCA) through Minitab 16 software. It is a statistical procedure that uses an orthogonal linear transformation that transforms the data to a new coordinate system.

3 Result and discussion
3.1 Result
Biochemical composition of aerial parts (leaves, floral, and apical buds) of *C. procera* varied among organs (floral, apical buds, and leaves) as well as climate condition and the lunar cycle. Plants were selected from the same habitat thus parameter changes are assumed to be uniform. (Table1: Meteorological report of *Calotropis Procera*) shows the meteorological data for a collection of floral and seeds of *C. procera* during the lunar and seasonal cycle. Sunrise, sunset, moonrise, and moonset times were noted according to www.Timeanddate.com.

C. procera leaves in the summer season at FM (PSFM) exhibited highest carbohydrate content (11.55 ± 0.0 mg g^{-1}) as compared to floral and buds parts. Floral showed maximum TCC content (11.69 ± 0.04 mg g^{-1}) in rainy season at NM (PRNM) followed by leaves and buds. A drastic increase of total carbohydrate content was observed in all 3 plant parts from NM to FM in all seasons except rainy season.

The results showed that the maximum protein content (TPRC) was recorded during winter season at FM (PWFM) in leaves (226 ± 0.39 mg g^{-1}) followed by buds (157.56 ± 0.49 mg g^{-1}) while maximum protein content was recorded in floral (248.67 ± 0.23 mg g^{-1}) during summer at FM (PSFM). Protein content was raised from NM to FM in all 3 plant parts during all 3 seasons except in buds during summer season (PSNM (137.07 ± 0.64 mg g^{-1}) – PSFM (128.27 ± 0.33 mg g^{-1}).

Flavanoid content, as a secondary metabolite, attained its maximum value during winter season at FM (PWFM) in all 3 plant parts. Leaves had a greater value (76.89 ± 1.02 mg g^{-1}) than flower (41.17 ± 1.76 mg g^{-1}) and buds (38.30 ± 0.61 mg g^{-1}) while minimum content observed in rainy season at NM (PRNM) in floral (6.80±0.10mg g^{-1}) and buds (1.00 ± 0.50) whereas summer season (PSFM) in leaves. It showed that there was an extensively increased flavanoid content in buds from NM to FM during all 3 seasons while decreased in leaves and floral in summer.

Total phenolic content was highest during winter season at NM (PWNM) in leaves (84.41 ± 1.09 mg g^{-1}) followed by floral (74.11 ± 0.67 mg g^{-1}) and then in buds (50.14 ± 0.12 mg g^{-1}). TPC content of *C. procera* was raised from NM to FM during summer and rainy season in leaves and floral and fall in winter (PWNM - PWFM) season while bud showed significantly elevated TPC content from NM to FM in all seasons.

The present study revealed that tannin content (TTC) exhibited highest concentration in different seasons in all plant parts; in floral (6.80 ± 0.20 mg g^{-1}) at FM in rainy, in buds (9.63 ± 0.55 mg g^{-1}) at FM in summer and leaves (6.43 ± 0.12 mg g^{-1}) at NM in winter. A slight difference was observed in all seasons from NM to FM.

Furthermore, the main secondary metabolite of *C. procera* i.e c. glycosides attained its maximum value in summer season at NM (PRNM) in leaves (80.67 ± 1.44 mg g^{-1}) compare to buds (79.52 ± 0.50 mg g^{-1}) at FM and flower (62.34 ± 0.40 mg g^{-1}) at NM. It was observed that cardiac glycoside content was minimum in rainy season in all plant parts. Cardiac glycoside content was raised from NM to FM in summer and rainy in leaves, floral, and buds while decreased in winter. pH was optimum during winter in leaves (6.8) and floral (6.8) compare to buds (6.5) in rainy.

3.2 Multivariate Statistical Analysis (PCA)
The lunar and seasonal variability in physiochemical parameters of *C. procera* was investigated for all aerial part extracts. PCA results state the samples were well differentiated from each other as they lie in four different quadrants. Components (PC1 and PC2) were chosen based on analyzed data obtained from leaves of *C. procera* holding 81.1% of the total variability (Fig 1). PC1 accounted for 56.7% variability, which was positive association with TPC, TTC, TFC, and TPRC in winter season and showed the largest distribution with the correlation coefficients, 0.477, 0.423, 0.479 and 0.302 respectively. While PC2 noted for 24.4% variability that positively associated with pH in rainy season. Summer seasons (PSFM-PSNM) lie in the negative region. Multivariate analysis of physiochemical and seasonal - lunar based activities of *C. procera* buds is given in Fig 2. PC1 accounted for 51.5% variability and strong positive association with TFC, TPRC, and TCGC showing largest significant correlation coefficient, 0.510, 0.473, and 0.399 respectively. Similarly, PC2 noted for 26.6% variability closely associated with pH and TPC in rainy and winter season (PWFM and PWNM) respectively and revealed that winter samples were differentiated based on TPC only. Summer season (PSNM) distributes in a negative region based on TTC and TCC values with strong positive correlation with each other. PC1 and PC2 of *C. procera* floral show 81.3% of the total variability (Fig 3). PC1 accounted 52.9% while PC2 observed 28.5% variability. PC1 revealed that it is closely associated with TPC, TCC, TTC in winter (PWFM and PWNM) and rainy (PRNM) showed largest significantly correlation coefficient, 0.294, 0.500, 0.527 respectively. Similarly, PC2 showed a closely association with TFC (-0.721). PSFM did not show any variable distribution.

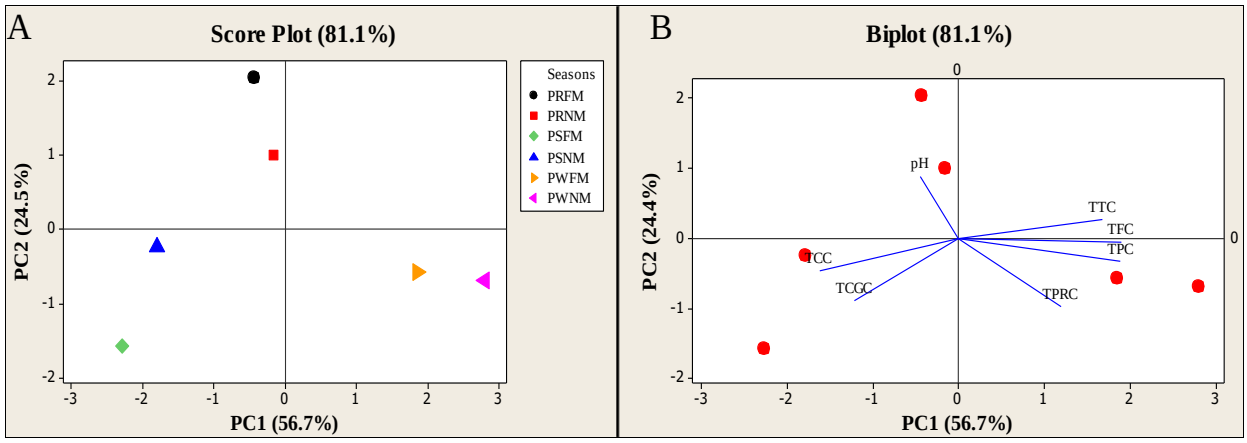

Fig 1: Principal component analysis based on phytochemical analysis and pH of *C. procera* leaves. (A) Score plot (81%) (B) PCA biplot (81%)

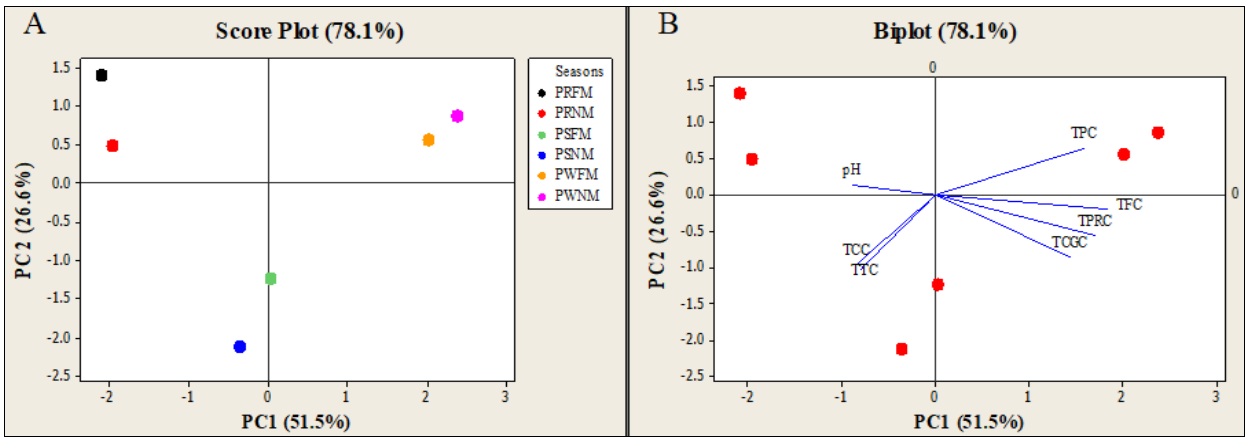

Fig 2: Principle component analysis based on phytochemical analysis and pH of *C.procera* Buds. (A) Score plot (78.1%) (B) PCA biplot (78.1%)

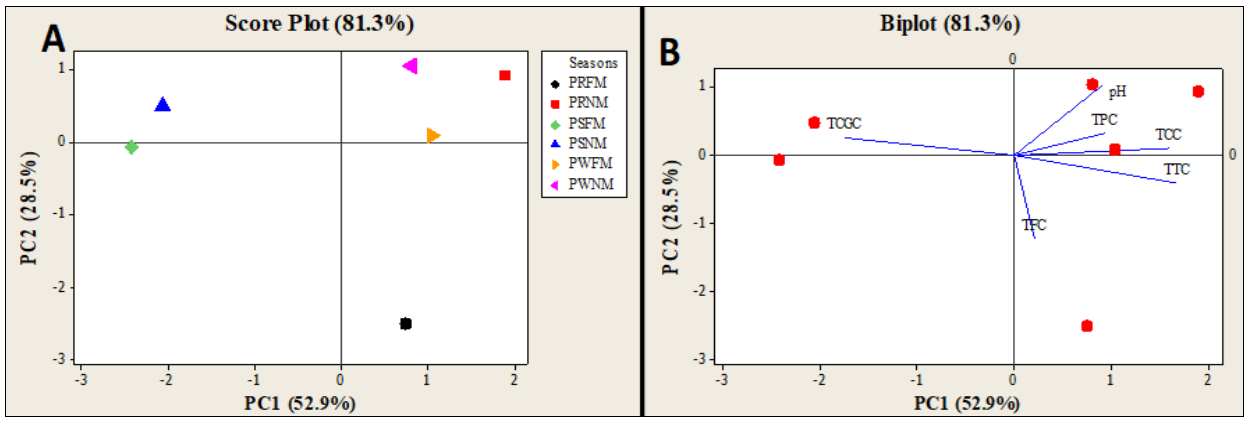

Fig 3: Principle component analysis based on phytochemical analysis and pH of *C.procera* Floral. (A) Score plot (81.3%) (B) PCA biplot (81.3%)

3.3 *C. procera* leaves, buds and floral new moon

Principle component analysis of New moon samples revealed that PC1 and PC2 of aerial parts demonstrate about 100% total variability (Fig 4). PC1 and PC2 of leaves New Moon accounted for 74.1% and 25.9% variability in winter and summer season showed close association with TPC, TFC, TTC and TCGC, TPRC respectively. TCC lied in a negative region in rainy NM. Multivariate analysis of *C. procera* buds showed that PC1 and PC2 accounted for 60% and 40% variability. TFC, TPRC, TCGC were positively associated with each other on PC1 but did not show any seasonal variation, only TPC showed variability with 0.448 coefficient on PC1 in winter and TCC (-0.481) in summer. Similarly, TTC and pH were strong positively associated with PC2 in summer season. 3rd Arial part of *C. procera* i.e. floral part revealed PC1 accounted for 72.8% which was closely associated with pH in winter and TCC, TTC in summer lie on positive region, while TCGC in rainy lie on negative region showing maximum correlation coefficient -0.468 compared to other. PC2 accounted for 27.2% and is associated with TPC.

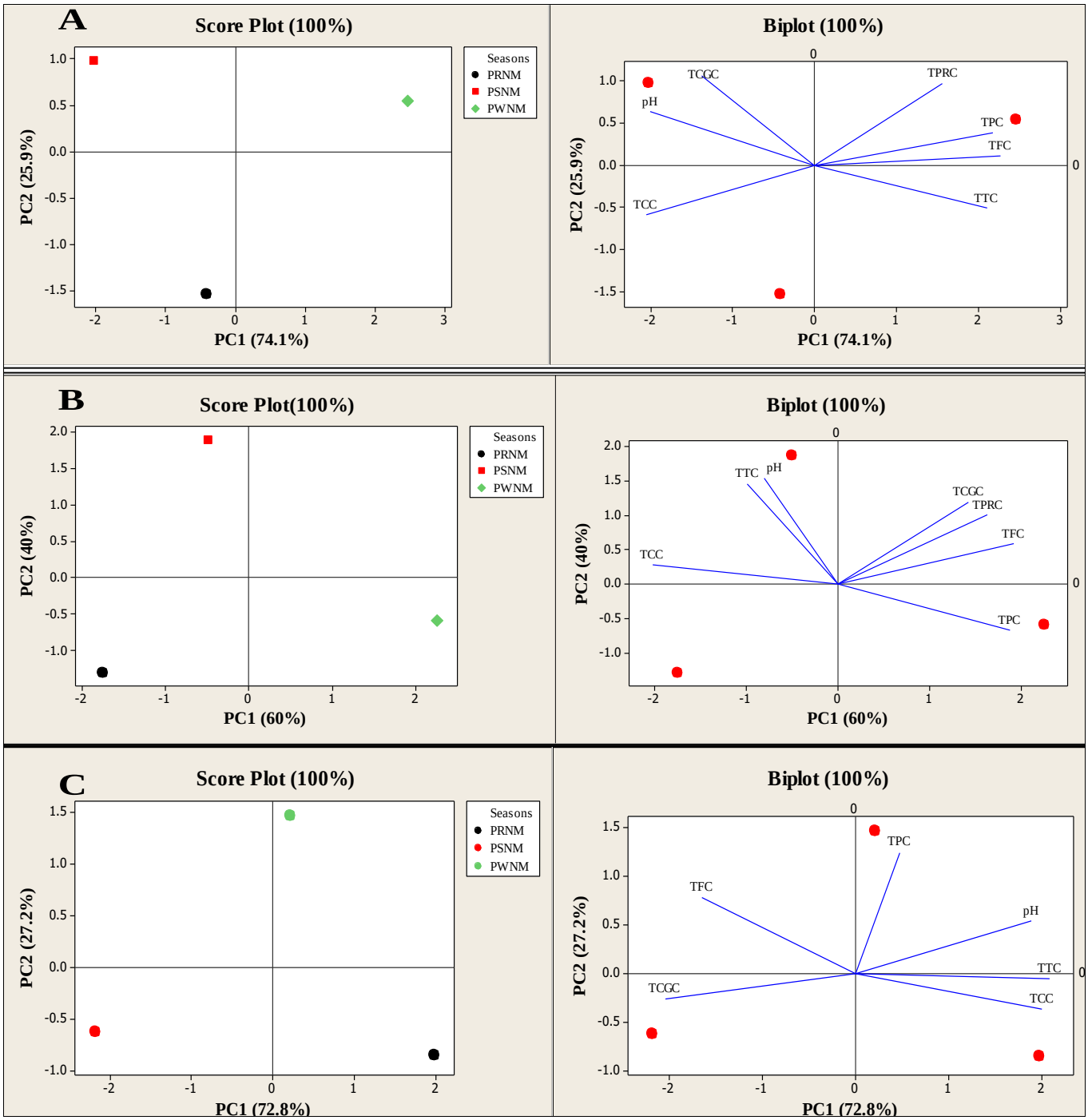

Fig 4: Principal component analysis based on phytochemical composition and pH of *C.procera* during new moon. Score plot (100%) and Biplot (100%) (A) Leaves of *C.procera*, (B) Apical buds of *C.procera*, (C) Floral of *C.procera*

3.4 *C. procera* leaves, bud, and floral full moon

Multivariate analysis of the full moon of aerial plant signified that PC1 and PC2 were about 100% of total variability (Fig 5). Leaves showed that PC1 and PC2 accounted for 66.8% and 33.2% variability respectively. PC1 was closely associated with TTC in rainy season and TFC, TPC in winter season lie on a positive region of principle component, whereas TCC and TCGC in summer season were laid on negative region of principle component. PC2 was significantly associated with pH in rainy showing a correlation coefficient, 0.482. PCA of buds explained that PC1 was significantly associated with TCGC and TPRC in summer season and TFC in winter season whereas TCC, TPC, pH (on negative region) were associated with PC2 in summer, winter, and rainy seasons respectively. Furthermore, the colorful part, floral revealed PC1 was closely associated with TPC, TCC during winter season, TTC in rainy season and TCGC in summer season lied on positive region showing largest distribution with correlation coefficient, 0.410, 0.499, 0.455 and -0.471 respectively. TFC and pH were significantly associated with PC2 in rainy and winter respectively.

Fig 6 showed the phytochemical comparison of Leaves, Buds and Floral extracts with seasonal and lunar cycle. Total cardiac glycoside content (Fig: 3 E) was higher in buds and leaves and nearby maximum in Floral. While tannin content (Fig: 6 F) was higher in buds > floral > leaves. TCC, TFC and TPC were higher in leaves and TPC, TCC content were higher in floral parts. Hence, different organs pronounced different phytochemical content.

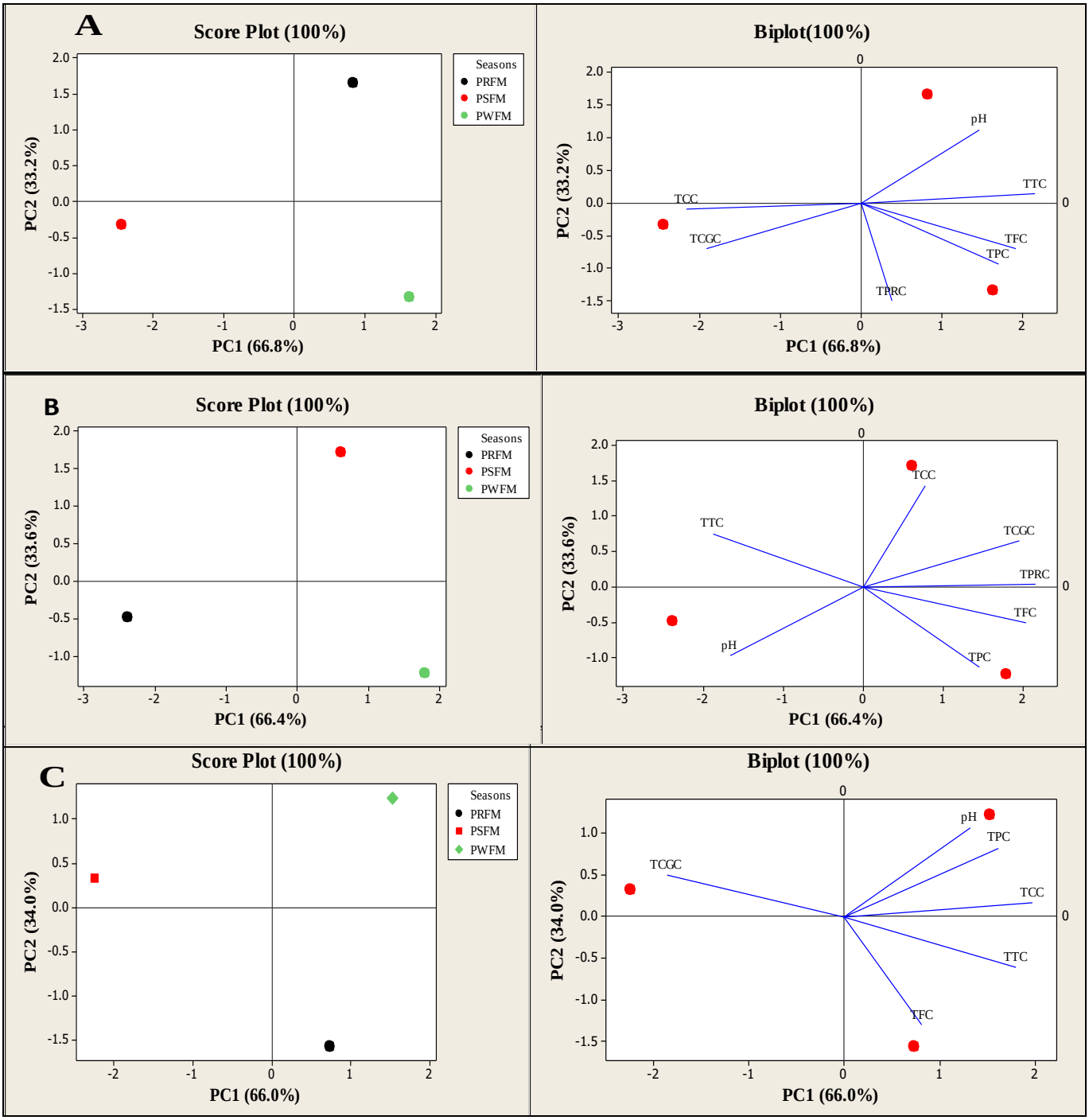

Fig 5: principal component analysis based on phytochemical composition and pH of *C.procera* during *Full* Moon. Score plot (100%) and Biplot (100%) (A) Leaves of *C.procera*, (B) Apical Buds of *C.procera*, (C) Floral of *C.procera*

4. Discussion

Medicinal plants are the heart of this universe which has prime importance in terms of agriculture and medicine. The phytoconstituents produced in the parts of plants play a significant role in many ways like; used as a plant protection and as an ethno-medicinal drug. Different organ would have different physiology and function and hence would have variability in the content of phytochemicals (Srivastava *et al.*, 2012) [16]. Plant primary metabolites are playing a role as a building block. Biosynthesis and accumulation of phytoconstituents are not depended only on genetic factor but largely depends on external biotic and abiotic factors also, such as collection practice, day length, night length, altitude, seasonal variation, environmental factors. This improves the quality and quantity of herbal drug that directly prevents the exploitation and enhance cultivation practice having minimum side effects, affordable cost and availability (Sharma *et al.*, 2013) [17]. There are numerous studies reported based on various environmental factors but not a single study is accessible based on the lunar cycle with seasonal variation except on *withania somnifera* [9]. These results support the seasonal, lunar and aerial plant parts collection for efficacy and pure drug.

In present investigation, carbohydrates ranged between 0.1 to 1.1% and 0.2 - 0.6% in leaves and buds respectively with maximum at FM summer while 0.1 - 0.1% in floral at NM in rainy. The accumulation of carbohydrate may be due to their utilization in various metabolic events, acts as molecule signals as well as regulating different genes which are involved in photosynthesis and osmolysis (Rosa *et al.*,2009) [18]. Protein was maximum in all parts of plant amongst all investigated bioactive constituents of *C. procera*.

Carbohydrate was next to the protein indicating the decrease in carbohydrate content may be due to the elevating in other metabolite content (Musa *et al.,* 2011) [19]. Protein content exhibited great variation along with lunar cycle and seasons. Maximum protein content (TPRC) recorded in a range of 24% in floral during summer at FM followed by 22% in leaves and a minimum of 15% in buds during winter at FM. The current study showed TPRC (24%) was maximum in leaves than reported by Kalita (23.94%) in same species (Kalita *et al.*, 2004) [20]. It was observed that the TPRC content had a significant increase from NM to FM during winter (PWNM - PWFM) in all 3 parts, while in summer moderate rise in leaves, a slight increase in floral and decrease in buds whereas declining in leaves, increase in buds and floral in rainy. Overall TPRC content was minimum in all parts during rainy season indicating moisture in the atmosphere may decline the TPRC content.

However, the scavenging properties of *C. procera* plant parts are associated with polyphenolic content (phenol and flavanoid), which can combat harmful free radicals and oxidative stress which damage DNA, which can lead to many degenerative diseases such as parkinson's, Alziemer's disease and other pharmacological activity like antidiabetic, anti-inflammatory, antiviral, antibacterial, etc. Polyphenols exert these effects as antioxidants, chelators of a divalent cation, and free radical scavengers [12]. These may be due to they hold a hydroxyl group. In present findings, Flavanoid content was maximum and ranged between 4.5 - 7.7% in leaves which was in agreement to study reported by Surveswaran with gigantea species, 1.1% - 4.1% in floral which was remarkably low than reported by Surveswaran in different spp. (Sureswaran *et al.*, 2010) [21], and 0.1 - 3.8% in buds during winter at FM (PWFM). Leaves attained maximum TFC indicating it promote physiological survival and protection to the plant and presence of TFC in flower is to provide colors for attraction, taste and protection of vitamins and enzymes (Kumar *et al.*, 2013) [22]. Flavanoid content extensively increased from NM to FM during winter and rainy season and decreased in summer in all plant parts. An increasing concentration of TFC was observed at full moon indicating the influences of the lunar phase. Presences of TFC content in buds during rainy season were almost nil in new and full moon due to high humidity and large amount of groundwater during rainy season.

Furthermore, total phenolic content was highest, and range between 1.1 - 8.4% in leaves, 1.8 - 7.4% in floral during winter at NM and buds attained 0.9 - 5.0% TPC content and maximum in winter at NM. Moreover, during summer at NM, TPC content was minimum, maybe due to lower humidity and higher temperature. There was a minimum variation in TPC content during the summer new moon and full moon in buds indicates some sort of nutritional stress. Authors reported to exhibit different level of phenolic content in leaves and flower i.e. 14 mg g^{-1} and 6.7 mg g^{-1} respectively which was comparatively minimum than current finding; TPC content in leaves and florals during winter season (78.50±1.73 mg g^{-1}) and (74.11±0.67 mg g^{-1}) respectively [16] and in agreement with Ahmed *et al*. (Ahmed *et al.*, 2018) [23]. Values of summer and winter apical buds of *C. procera* were in agreement with Alali *et al*. (Alali *et al.*, 2007) [24]. A previous study reported that the winter is the best season for accumulation of phenol and flavanoid content may be due to phenolic compounds play a critical protective role during moisture deprived season like winter season (Mukherjee, 2019) [25]. Most of the plants complete their life cycle i.e cellular process during season. Higher concentration of Phenolics and flavanoids, may be due to maturity of plants and low temperature level during winter season [26]. Moreover, the tannin content ranged maximum in between 0.5% - 0.96% in buds at summer NM, followed by moderate in floral, 0.14% - 0.68% in rainy at FM and minimum in leaves 0.14% - 0.64% in winter season at NM. These results are in agreement with the previous study reported that tannin content was maximum in leaves than flower with minor variation. Tannin was found to be half in content during winter, slightly fall- in leaves during rainy, decreased during summer (PSNM - PSFM) in buds and same in floral from NM to FM. While TTC content increased during summer in leaves, during winter and rainy in buds and floral extracts. Tannins are oligomeric and polymeric polyphenol and can precipitate the proteins, gelatin, alkaloids, etc, and exhibit numerous biological and pharmaceutical activities. Furthermore, the cardiac glycosides (CG) are secondary metabolite present only in milky weeds which can increase the cardiac contractile force with congestive heart failure (Ravi *et al.*, 2020) [26]. Although *C. procera* is toxic in nature and an evergreen plant, it has a capability to treat numerous disease and high in demand in medicinal and agriculture area. CG content was found to be maximum during summer (PSFM and PSNM) in buds (79.52 ± 0.50 mg g^{-1}), floral (67.83 ± 0.41 mg g^{-1}), and leaves (80.67 ± 1.44 mg g^{-1}) while minimum during PRNM in leaves (24.39 ± 0.47mg g^{-1}), and floral (20.33 ± 0.29 mg g^{-1}) and during rainy season (26.33 ± 1.26 mg g^{-1}) in buds. In current research it was observed that buds showed remarkably highest value in summer which was in agreement with earlier reported in *Asclepias eriocarpa* plant as well as higher CG content in buds than florals and leaves (Nelson *et al.*, 1981) [27]. In current study, the pH was optimum during winter (PWNM) and rainy (PRNM) in leaves (6.8 and 6.6) and floral (6.8), during rainy (PRFM) in buds (6.5).

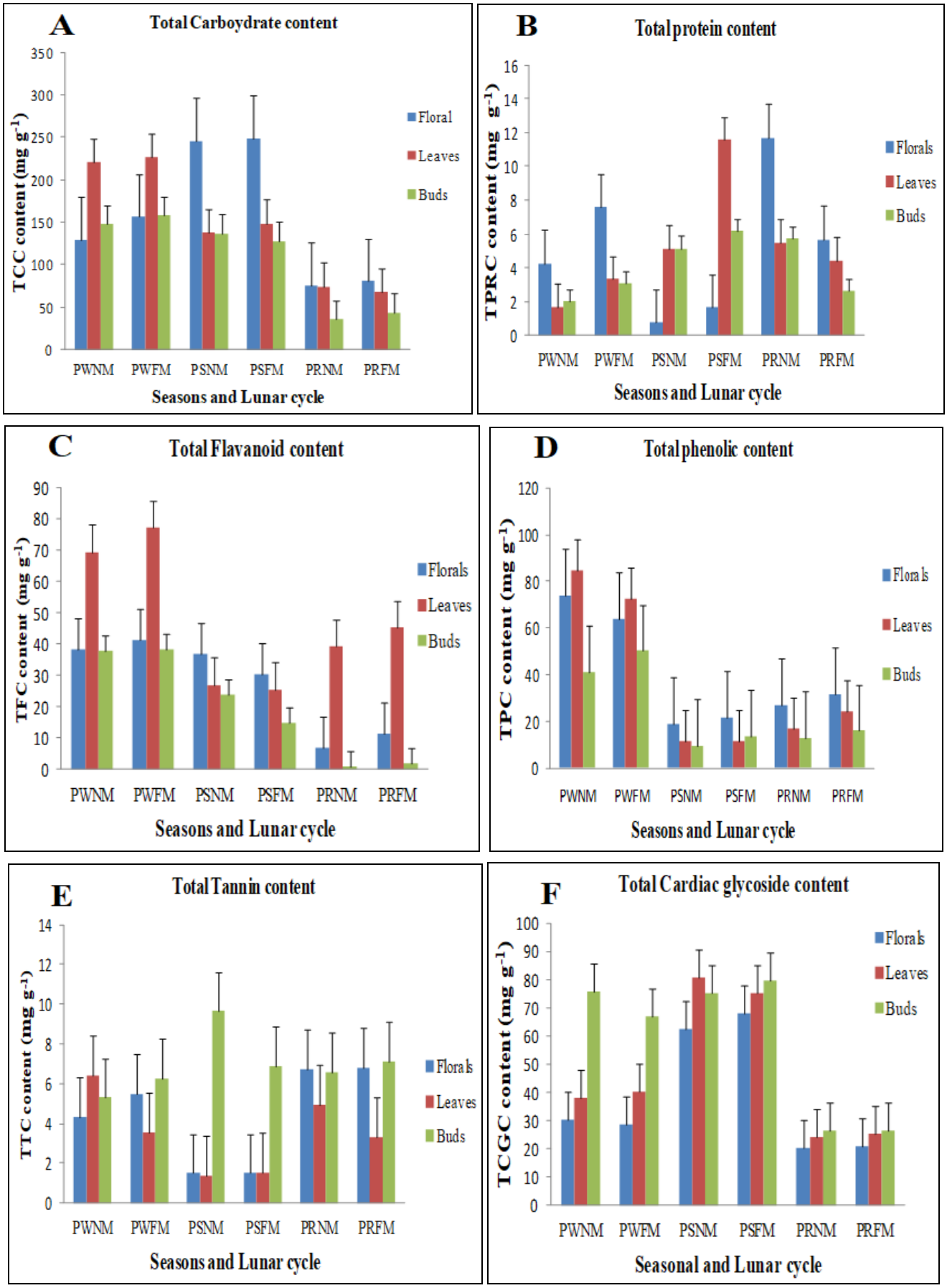

Fig 6: Physiochemical comparison of *C. procera* leaves, floral and apical buds extract based on seasonal and lunar cycle (A) Total carbohydrate content, B) Total protein content, C) Total Flavanoid content, d) Total phenolics content, E) Total cardiac glycoside, F)Total Tannin content

4.1 Multivariate Statistical Analysis (PCA)

All the variables were equally distributed in 4 quadrants. The Examination of parameters of leaves, buds, and floral extract of *C. procera* explained that component1 (PC1) was dominated by TPC and TPRC in winter season (FM and NM) whereas component 2 (PC2) was dominated by pH ((+, -) region) in rainy, and TCC and TCGC (-,-) in summer season.

In buds, component 1(PC1) was dominated by TPC (in

winter), TCC (summer) and pH (rainy) while in floral, component 1(PC1) was dominated by pH, TPC, TCC in winter (FM and NM) and rainy (NM), TTC in rainy (FM), and TCGC in summer whereas component 2 (PC2) was dominated by TFC in rainy season. TPC was quantitatively distinguished but distributed in same quadrant. It was higher in winter than rainy and TCC was higher in rainy than winter. These Phytochemicals are maximum synthesized mainly in the specific season. Hence, it confirmed that the periodicity of three seasons impacted on extractive principles of *C. procera* [26].

4.2 *C. procera* leaves, buds, and floral New moon

Principle component analysis of new moon samples revealed Component 1 (PC1), as dominated by TFC, TPC, and TCC during winter in leaves while TPRC and TCC in winter and summer in buds whereas TTC, TCC, and TCGC during rainy and summer in floral respectively. Component 2 (PC2) was dominated by TPRC during winter, TCGC in summer in leaves; TTC, pH during summer in buds and TPC in winter and TCGC during summer in floral part. It was noticed that some Phytochemicals were synthesizing excessively on new moon days.

4.3 *C. procera* leaves, buds, and floral full moon

Principle component analysis of full moon samples revealed Component 1 (PC1) was dominated by TTC in rainy, TCC and TCGC on negative quadrant during summer and TFC, TPC during winter in leaves; while TPRC, TCGC and TFC during summer and winter respectively in buds and TPC, TTC and TCGC during winter, rainy and summer respectively. Component 2 was dominated by TPRC during winter in leaves, while TCC and TPC in summer and winter respectively whereas TFC and pH in rainy and winter in floral part. It is inferred that productions of phytochemicals was significant higher during full moon days.

Fig 4 shows the phytochemical comparison of Leaves, Buds and Floral extracts based on seasonal and lunar cycle. Total cardiac glycoside content (Fig 4(E)) was higher in buds and leaves and maximum in floral. While tannin content (Fig 4(F)) was higher in buds > floral > leaves. TCC, TFC and TPC were higher in leaves and TPC, TCC content were higher in floral part in accordance with different organ producing different phytochemical content.

Amongst all the season, the night length of winter season days was more than the summer and the rainy season. Hours of day length of the summer season are slightly more than the rainy season but less than winter from both seasons. When compared to New moon days, Full moon days received more light which may be the key factor responsible for more photosynthetic activity depends on sunlight, anabolism activity, and moonlight (up to 99.5%) [9]. Besides the genetic factors and environmental factors, average temperature and day light length during the period prior to harvest could be determined.

Table 1: Meteorological report for *Calotropis procera*

Season	Date	Lunar Phase	Sun-rise(am)	Sun-set(pm)	Moon rise	Moon set	Moon rise	DN(h)	NL(h)	Illumination	Rainfall (mm)
Winter	14/11/2016	FM	06:47	17:57		06:14	18:04	11:09:54	12:51	99.30%	0
	29/11/2016	NM	06:56	17:55	06:34	18:03		10:58:45	13:02	0.20%	
Summer	22/05/2015	FM	05:58	19:11		06:20	19:38	13:13:28	10:47	99.80%	±0.6
	05/06/2016	NM	05:56	19:17	06:06	19:33		13:21:14	10:39	0.20%	
Rainy	03/08/2016	NM	06:12	19:15	06:28	19:36		13:03:05	10:57	0.30%	±303.9
	18/08/2016	FM	06:17	19:06		06:00	19:06	12:48:17	11:12	99.50%	

FM: Full Moon, NM: No Moon, DL: Day Length, NL: Night Length
Courtesy: Irrigation Division, Government of Gujarat, Navsari

5. Conclusion

The study explored the significant variation in production of *C. procera* phytoconstituents during different seasons as well as lunar cycle by collecting the sample during full moon and new moon. *C. procera* is widely used as an ethano medicinal plant from folk use (cough, eczema, abortion, convulsion) to pharmacological use (antidiabetic, anticancer, arthritis, antibacterial, analgesic activities). These studies are helpful in finding the best collection season and time to procure a best quality drug with less exploitation of plant based on the compound. There was a drastic variation in synthesis of phytoconstituents quantitatively, due to the environmental factors like temperature, rain, day length, night length and lunar cycle. Present finding demonstrated that the phytochemicals were found quantitatively more during full moon compared to new moon except few metabolites were more active during new moon. Winter (for protein, flavanoids, phenolics, and tannin), and summer (carbohydrate and cardiac glycoside) season were favorable for leaves, summer (protein), winter (flavanoids, phenolics), rainy (carbohydrate, tannins) for floral part, and winter (protein, flavanoids, and phenolics), summer (carbohydrate, tannin and cardiac glycoside) seasons were favorable for apical buds. In nutshell, the concept of collection of plant parts according to lunar cycle and seasons has the strongest potential to maximize the quality, quantity, efficacy and purity of which may useful in pharmaceutical area and minimize the flora exploitation. Further research on isolation and identification of compound may open the door towards innovative research.

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7. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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