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High performance liquid chromatographic analysis of phenolic compounds and their antioxidant properties from different cultivars of *Cyamopsis tetragonaloba* (L.) Taub

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ABSTRACT

Guar (*Cyamopsis tetragonaloba* (L.) Taub) is an annual legume plant and an important source of guar gum, which has great industrial and medicinal value due to phenolic compounds. Thus the consumption of guar and its products has been associated with a reduction in the risk of contracting some types of cancer and other chronic diseases. Phenolic compounds or polyphenols act as antioxidants with mechanisms involving both free radical scavenging and metal chelation. Thus, fifteen different cultivars of the cluster bean leaves i.e. *Cyamopsis tetragonaloba* (L.) Taub were evaluated for their content of phenolic acids and flavonoids using optimized high performance liquid chromatography (HPLC) with UV detection. These cultivars were also analyzed for antioxidant property using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) activity. Rajasthan guar cultivar-1031 (RGC-1031) showed higher phenolic contents along with better antioxidant property. These results were also confirmed with HPLC where ten phenolic/flavonoid compounds were detected and quantified at varying levels in leaves of different cultivars of guar. Polyphenols such as sinapic acid, cholorogenic acid, caffeic acid, gallic acids and ferulic acid were detected with binary mode of pumps within 10 min with a flow rate of 1.0 mL/min where as flavanoids like kaempferol and myricetin were also identified showing variations among all cultivars. The total phenolic contents and total flavonoid contents in all cultivar were in the range 60.03 to 204.67 mg GAE/g and 4.26 to 12.43 mg QE/g, respectively. In particular, the cultivar RGC-1031 showed the highest content while Local-Selection showed the least content of the total polyphenols. Kaempferol was found to be major constituent in the studied guar cultivars than other phenolics. The extensive variability was observed in polyphenols among fifteen cultivars of guar. The Principal component analysis (PCA) showed that Local-Selection contained higher kaempferol and myricetin, while Pusa Selection-1 recorded higher cholorogenic acid and kaempferol.

1. Introduction

Polyphenolic compounds are commonly found in both edible and inedible plants [1]. Polyphenols i.e. flavonoids and phenolic acids, which are phenolic compounds widely, exist in fruits, vegetables [2], whole grains [3] and other plants with several individual compounds known [1,4,5–9]. These are the group of highly hydroxylated phenolic compounds present in plants and products of plant origin and act as inhibitors on the growth of microorganisms. They are derived from phenylalanine and tyrosine and generally occur as glycosylated derivatives with antioxidant activity and inhibit the oxidation of low density lipoproteins [10]. They possess chemical structures favoring antioxidant actions like scavenging radicals and chelating redox-active metals and thus known to act as antioxidants in vivo [11]. Phenolic compounds i.e. flavonoids and phenolic acids, have been reported to have many other beneficial health effects such as antioxidant activity, anti-inflammation, antibacterial, antiviral, antiallergenic reaction, antimutagenic and anticancer properties [3,12]. Antioxidants are the molecules that delay or prevent oxidation of an oxidizable substrate by producing free radicals and have multiple functions in biological systems, including defense against oxidative damage and exerting beneficial effects in major signaling pathways of cells [13]. Therefore, their intake contributes to the prevention of cardiovascular diseases,

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cancers, osteoporosis, neuro-degenerative diseases and diabetes mellitus [11].

Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Thus, phenolic compounds or polyphenols, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. Their contents in plant foods are largely influenced by genetic factors and environmental conditions, for example, plant nutrient availability, climate, pathogen infection and pest attack [12].

Flavonoids are the naturally occurring polyphenols representing one of the most prevalent classes of compounds in medicinal herbs such as *Silybum marianum*, *Alpinia officinarum*, *Hypericum perforatum* and also in vegetables, nuts, fruits and beverages such as coffee, tea and red wine [14]. Epidemiological studies have shown the protective role of flavonoids against various cancers and particularly hormone related cancers [15]. Flavonoids are also the most usual group of phenols in human diet. Considerable evidence indicates that some of the protective effects of phenols on fruits and vegetables may be due to flavonoids [16]. Flavonoids are potent free radical scavengers with antioxidant capacity, tested under in vitro [17] and in vivo conditions [18]. Polyphenols, present in a variety of plants, are utilized as important components of both human and animal diets [19–21]. Therefore, it is extremely important to determine the phenolic compounds i.e. flavonoids or polyphenols in vegetable, feed and fodder along with their antioxidant properties.

Cluster bean is commonly known as guar [*Cyamopsis tetragonoloba* (L.) Taub]. It is an important indigenous, annual and self-pollinated kharif legume in India grown for feed, green fodder, vegetable, green manuring, and grain purposes [22,23]. It is an important source of nutrition to animals and humans, and being extremely drought resistant, grown in semiarid regions where most other plants perish. In addition, it is widely used as raw material for a wide range of industrial products. Cluster bean is a potential source of protein to meet the staggering nutritional demand. It is a cash crop due to its application in various industries as it contains galactomannan gum which has diversified industrial uses such as in food, pharmaceutical, cosmetics, textiles, paper, explosives, mining, tobacco, oil, gas and petroleum industries [22,23]. Consequently, cluster bean being a high-value crop, provides consistent income to farmers and with it India earns several thousand crores of rupees as foreign exchange from export of guar gum and its derivatives [23]. In addition to social and dietary uses guar is also used as remedies against various diseases in traditional system of medicine as its green pods and gums are used for curing diabetes, leaves for night blindness while seeds are used against small pox [23]. However, only one study has been reported on the toxicity of polyphenols in the seeds and leaves of guar [3]. Thus, the chemical profiling is important for characterization of different cultivars of cluster bean but there is no report on the phenolic compounds in its cultivars. This information may also be useful to breeding programmes for improving guar seed quality. Furthermore, the phytochemical analysis of guar may expand its nutraceutical and pharmaceutical utilization. Therefore, it was extremely important to analyze the phenolic compounds in different cultivars of cluster bean for their characterization and is reported in the present study.

2. Material and methods

2.1. Reagents

Standard phenolic acids were obtained from HiMedia and Sigma-Aldrich companies. The solvents (methanol, ethanol, orthophosphoric acid, glacial acetic acid, acetonitrile, distilled water, etc.) used during HPLC analysis were of the HPLC grade (Rankem). DPPH was obtained from Sisco Research Laboratories Pvt. Ltd. (India). Phenolic acid standards were accurately weighted and dissolved in methanol for preparing 1000 μg/mL stock solutions. The solutions were further diluted (100 μg/mL) to obtain the working standards.

2.2. Sampling of plant materials, sample pre-treatment and storage

Six major cluster bean growing states of India i.e. Rajasthan, Gujarat, Haryana, Punjab, Madhya Pradesh (MP) and Utter Pradesh (UP) were selected for collection of seed samples as the soil and climatic conditions of these states are favorable for production of guar. The different cultivars of the cluster bean are listed in Table 1. Guar seeds (one kg) were collected from Krishi Vigyan Kendra (KVK) of these selected states. Seeds were placed in Ziploc-type freezer bags and brought to the lab at Banasthali. These were germinated in the pots at 25 °C. The pots were placed in greenhouse under natural light at 30/20 °C (day/night) and a relative humidity of 60/70% (day/night). The experiments were designed with three replicates of each cultivar. Matured fresh and healthy leaves free from disease were collected at six weeks after transplanting during the month of April 2010 for the extraction of phenolic compounds. Matured leaves of guar were washed thoroughly 2–3 times with running tap water, doubled distilled water (DDW) and air dried. This was followed by lyophilization to produce dried powder extract. The freeze-dried extract was re-dissolved in 70% methanol.

2.3. Preparation of plant extracts

Air-dried powdered samples with 70% methanol were extracted using slightly modified reported method [24,25]. The extract was then homogenized by using high efficiency homogenizer (IKA® Ultra Turrax T25) and kept for three days on shaker at room temperature. Extract was centrifuged (DuPont, model Sorvall RC-5C) to obtain the supernatant extract. The residues were re-extracted under same conditions. Supernatants were pooled, combined and evaporated with a rotary evaporator (Nutronix, Jain Brothers, India) at ≤50 °C. All the extracts were stored at 4 °C until analyzed. The extraction was performed in triplicates.

2.4. Quantification total phenolic & flavonoid contents

The total phenolic contents (TPC) of the extract from different samples material was assayed using Folin-Ciocalteu (FC) phenol reagent [26] and expressed as gallic acid equivalent (GAE). To an aliquot of the extract (0.5 mL), 8.5 mL of DDW and 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min of incubation, 1 mL of 25% sodium carbonate was added, mixed well and the reaction mixture was incubated at room temperature for 1 h. Then absorbance was measured at 725 nm using a UV–visible spectrophotometer (UV-2600, Thermo Scientific Spectroscan).

The total flavonoid content (TFC) of the extract was determined calorimetrically [27]. The extracted aliquot (0.2 mL) was added to different test tubes containing 0.1 mL of 10% $Al(NO_3)_3$ (w/v), 0.1 mL of 1 M potassium acetate and 4.6 mL ethanol (80%). After incubation for 40 min at 26 °C, the absorbance was measured against reagent blank at 415 nm. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of samples.

Table 1
Different cultivars of cluster bean collected from different states of India.

States	Cultivars of cluster bean	Climatic zone
Rajasthan	RGC-936, RGC-1002, RGC-1003, RGC-1031, RGC-1017, RGC-1066	Arid/Semi-arid
Haryana	HG-365, Priya-151	Semi-arid
Gujarat	Swati-55, Jyoti-555	Semi-arid
Utter Pradesh	Pusa-Navbhar, Neelam-51, PNB	Semi-arid
Punjab	Jyoti-55, Local-Selection	Humid subtropical
Madhya Pradesh	Pusa Selection I	Semi-arid

2.5. DPPH scavenging assay

The DPPH solution was used for determination of free radical scavenging of extracts following method reported by Chang et al. [28]. Briefly, 10 μL of each sample (10 mg/mL) was mixed with 90 μL of 50 mM tris(hydroxymethyl)aminomethane–HCl buffer (pH 7.4) and 200 μL of 0.1 mM DPPH-methanol solution. After 30 min of incubation at 25 °C, the absorbance of the solution measured was measured at 517 nm. The experiment was performed in triplicate. The inhibition ratio (%) was calculated according to the equation: Inhibition (%) = [(absorbance of control − absorbance of sample) / absorbance of control] × 100. The results were reported in RC50 value which indicates the concentration of the tested sample required to reduce the free radical concentration by 50%.

2.6. Identification & quantification of phenolic compounds

The quantitative and qualitative analysis of phenolic acids and flavonoids in guar leaf extract samples was carried out by high performance liquid chromatography (HPLC). The extracts were concentrated to 50% using concentrator and filtered through 0.4 μm Millipore filters device. The filtered samples were injected into a 20 μL loop injection valve of HPLC (Shimadzu LC-10A) equipped with SCL-10 AVP analog pump and SPD 10 AV detector connected to the system processor. The maximum pressure 400 kgf/cm^2 and the minimum 0.0 kgf/cm^2 were maintained. The HPLC of solvent was run at 320 nm using a reverse phase C-18 column. The conditions were optimized for separating out phenolic compounds from methanolic extract of different cultivars of cluster bean through HPLC. Different standard solutions of the phenolic acids were prepared using vacuum concentrator (Heto VR-1 Denmark). The mobile phase consisted of solvent A as orthophosphoric acid (1.5%) and solvent B which was orthophosphoric acid, glacial acetic acid and acetonitrile as 1.5, 20 and 25%, respectively. The flow rate was 1.0 mL/min while the 20 μL of sample was injected into injection loop. The chromatograms were processed using a Shimadzu-LC-10A connected with the PC. The maximum pressure of 400 kgf/cm^2 was maintained while solvent A:solvent B ratio was 30:70. Identification of the chromatographic peaks was performed by comparison of the individual retention time (RT) of the known standards of phenolic compounds as shown in Table 2. Seven phytoalexins i.e. polyphenols detected and determined in the present study were sinapic acid (SA), ferulic acid (FA), gallic acid (GA), chlorogenic acid (CGA), kaempferol (KP), myricetin (MY) and caffeic acid (CA). The chemical groups and structures of the determined polyphenols are shown in Table 2. Quantification of individual peak of the polyphenols was achieved by comparison to the sample internal standards.

2.7. Statistical analysis

Statistically significant differences ($p < 0.05$) among the means of total phenolic content, total flavonoid content and DPPH free radical scavenging activity were evaluated by analysis of variance and the mean compared by Duncan's Multiple Range Tests. The results were expressed as mean value ± standard deviation (SD). The principal component analyses (PCA) and hierarchical clustering were used to display the correlation between various phenolic compounds and their relationship with the different cultivars. The multivariate analysis was carried out using the SASJMP version 9.0 and SAS enterprise guide 4.0 software.

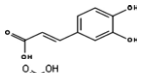
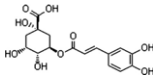
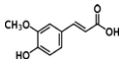
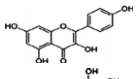
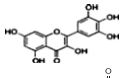
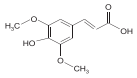
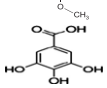
3. Results and discussion

Polyphenols form the largest group of compounds among natural antioxidants. The quantitative composition of individual fractions of phenolic compounds, even within one species, is very diverse and depends on many factors, such as the area of cultivation, maturity, climatic conditions during growth, and the harvest season and storage time after harvest [29]. Phenolic compounds are the most important group of secondary plant products that play an important role in reducing the susceptibility of a plant to pathogen [30]. The quantity and quality of phenolic compounds in plants have been used as criteria for identification of plants' varieties. For example, three species of *Rhodiola* and *Andrographis paniculata* were characterized on the basis of their phytochemical characteristics [31,32]. Thus, the study of secondary metabolites i.e. flavonoids, allied phenolic and polyphenolic compounds of higher plants have received considerable attention in recent years [1,3,4,6,33].

3.1. Phenolic contents in cultivars

The methanolic extracts of leaves of different cultivars were used for separation and quantification of different phenolic compounds by HPLC. The representative distributions of phenolic compounds in cluster bean leaves are shown in Fig. 1. The representative chromatograms clearly indicate the variation of different phenolic compounds in all the cultivars. The peaks for the phenolic compounds in the chromatograms were identified by their retention time of the standard phenolic compounds with the samples. The number of peaks in chromatograms gave the

Table 2
HPLC retention time of standard phenolic compounds and their structure.

RT (min)	Types	Chemical group	Chemical structure
4.33	Caffeic acid (CA)	Phenolic acid	
5.192	Chlorogenic acid (ClA)	Phenolic acid	
3.517	Ferulic acid (FA)	Phenolic acid	
7.508	Kaempferol (KP)	Flavonoid	
9.183	Myricetin (MY)	Flavonoid	
3.442	Sinapic acid (SA)	Phenolic acid	
3.692	Gallic acid (GA)	Phenolic acid	

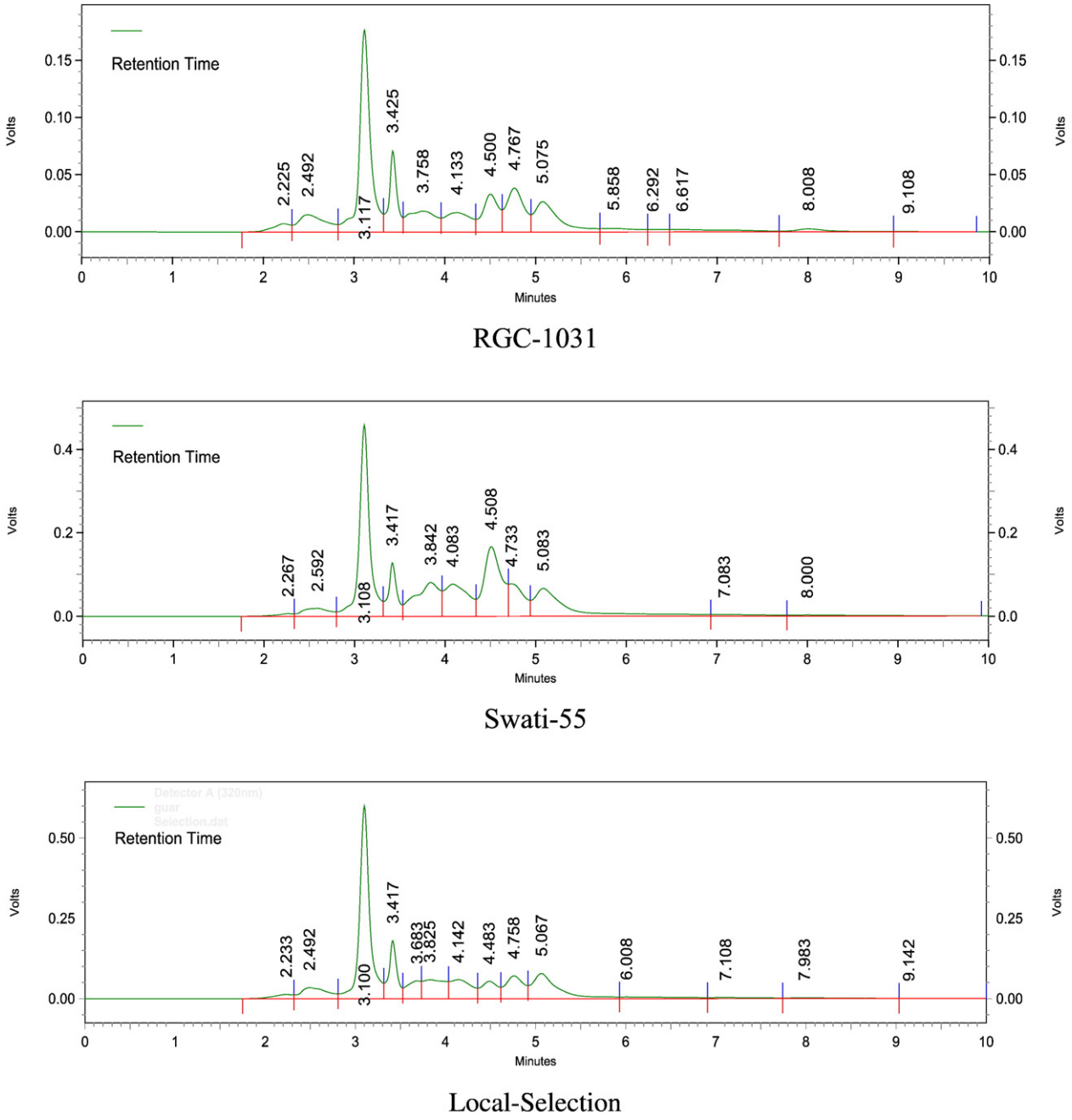


Fig. 1. Representative HPLC chromatograms of *Cyamopsis tetragonoloba* (L) Taub cultivars (RGC-1031, Swati-55 and Local-Selection) extracts using mobile phases with solvent A: orthophosphoric acid (1.5%) and solvent B: orthophosphoric acid, glacial acetic acid and acetonitrile (1.5%, 20% and 25%, respectively) showing the peaks of identified phenolic compounds with retention time (cf. Table 2).

number of phenolic compounds present in the sample while corresponding peak area determined their quantities. The major phenolic compounds determined in the leaves of different cultivars of cluster bean are shown in Table 3. It was found that CA, SA, and CGA were the most common phenolic present in almost all the extracts (Table 3).

The concentrations of each phenolic compound were calculated on the basis of peak area. It was observed that SA, CGA and CA were mostly present in all genotypes of cluster beans. The contents of different phenolic compounds in all cultivars in $\mu g \cdot gfw^{-1}$ are summarized in Table 3. The amount of CA in *Zizyphus mouritiana* has been reported to be 19 $\mu g \cdot gdw^{-1}$ [34]. Conversely, the lower amount of CA reported in pomegranates was 0.78 $\mu g \cdot L^{-1}$ [35]. Similarly, as shown in Table 3 the amount of CA in all cultivars of guar varied from 8.0 $\mu g \cdot gfw^{-1}$ in Priya-151 to 18.7 $\mu g \cdot gfw^{-1}$ in Pusa Selection I whereas FA was observed only in some cultivars and varied from 16.5 $\mu g \cdot gfw^{-1}$ in RGC-936 to the lowest 6.01 $\mu g \cdot gfw^{-1}$ in PNB. GA also varied in all cultivars of guar from the minimum 8.75 $\mu g \cdot gfw^{-1}$ in Local-Selection (Rajasthan) to the maximum 22.8 $\mu g \cdot gfw^{-1}$ in PNB (UP) but it was not detected in Pusa Selection I (MP) and Swati-55 (Gujarat) cultivars. In contrast to other polyphenols less variation was observed in case of SA as 5.6 $\mu g \cdot gfw^{-1}$ in RGC-936 and 7.87 $\mu g \cdot gfw^{-1}$ in Local-Selection. Similarly, CGA was present in the highest amount 25.9 $\mu g \cdot gfw^{-1}$ in Swati-55 and the lowest 16.8 $\mu g \cdot gfw^{-1}$ in RGC-1002 (Table 3).

As shown in Table 3, few cultivars showed higher concentrations of flavonoids i.e. KP and MY in comparison to other phenolics. KP was the lowest 22.4 $\mu g \cdot gfw^{-1}$ in HG-365 and highest 52.2 $\mu g \cdot gfw^{-1}$ in Pusa Selection I but it was not detected in cultivar of Rajasthan i.e. RGC-1066 and Swati-55 cultivar of Gujarat. These values are in agreement with

phenolic acids reported in different preparations of pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) [36]. Higher concentration of KP has been reported in guar leaves [3] as well as in guar seeds [37]. Thus, it was concluded that KP (52.20 $\mu g \cdot gfw^{-1}$) is the major constituent of these guar cultivars than other phenolics such as SA, CA, GA, FA, CGA and MY (Table 3).

The total phenolic contents (TPC) and total flavonoid contents (TFC) in leaf tissue of different cultivars of cluster bean from different states in India were in the range of 60.03 to 204.67 mg GAE/g and 4.26 to 12.43 mg QE/g, respectively as shown in Table 4. The highest level of total phenolics was recorded in RGC-1031 cultivar. On the other hand, the phenolic content was the lowest in Local Selection cultivar (Table 4).

The representative chromatograms shown in Fig. 1 clearly show that the peak with retention time of 3.10 ± 0.5 was the most common among fifteen samples. Area of this peak varied among all cultivars but it could not be identified as its retention time did not match with our available standards. Escarpa and Gonzalez [38] also indicated that such approach is viable for quantification of total phenolics. Clerivet et al. reported a distinct correlation between the degree of plant resistance and phenolics present in plant tissues [39]. A perusal of data in Table 4 clearly shows that TPC was the maximum in RGC-1031 in comparison to other cultivars. This observation confirms that RGC-1031 is more tolerant as compared to other guar genotypes in this study than other cultivars. By evaluation of resistance to *M. phaseolina* under artificially inoculated conditions in twenty five cluster bean genotypes has also been reported in literature [40]. Similar observation has also been reported by us [41].

3.2. Anti-oxidant study

To investigate the anti-oxidant activities of different cultivars of guar, the methanolic extracts were analyzed for the free radical scavenging activity. As shown in Table 4, methanolic extracts of different cultivars of guar displayed varying free radical scavenging activities. RGC-1031 showed the good anti-oxidant activity (RC50 = 22.5% inhibition, the concentration of the extract reducing 50% of the DPPH free radicals after 30 min) compared with other cultivars, supporting the fact that the polyphenolic constituents are responsible for the free radical scavenging activities in the cultivars. Kang et al. have shown that phenolic compounds act mainly as reducing agents, hydrogen donors and singlet-oxygen quenchers during anti-oxidant mechanisms [42]. This indicates that the high level of free radical scavenging activity in the extract of RGC-1031 might be medicated by the presence of high amount of phenolic compounds. In order to ensure the relationship between the level of phenolic compounds and free radical scavenging activity, the total phenol and flavonoid contents from methanolic extracts of guar was analyzed. As shown in Table 4, it can be concluded that RGC-1031 is the rich source of total phenolic content & total flavonoid content. Neelam-51, Jyoti-555 also contained a relatively higher level of phenolic compounds compared to that of other samples. Local-Selection was found to possess the lowest total phenolic content. In addition, total flavonoid levels proceeded in the order: RGC-1031 (4.26 mg QE/g), Neelam-51 (5.5 mg QE/g) Jyoti-555 (10.66 mg QE/g).

These findings indicated that the high amount of phenolic compounds in guar should be responsible for its free radical scavenging properties. However, Bondet et al. have shown that most phenolic compounds react slowly with DPPH, reaching a steady state in 1–6 h [43]. In addition, the color interference of DPPH with sample which contained anthocyanins results in the lowering of antioxidant activity [44]. This

Table 3
Major phenolic compounds determined in the leaves of different cultivars of cluster bean ($\mu g \cdot gfw^{-1}$).

Different cultivars	SA	FA	GA	CA	CGA	KP	MY	No. detected
RGC-936	5.60	16.50	16.70	17.00	17.60	28.90	26.40	7
RGC-1002	6.90	nd	8.75	17.10	16.80	29.30	12.30	6
RGC-1003	5.72	nd	16.20	14.90	17.00	27.70	14.30	6
RGC-1031	6.05	nd	16.70	17.70	19.70	41.60	nd	5
RGC-1017	6.01	nd	22.70	16.90	22.30	28.60	20.60	6
RGC-1066	5.65	nd	20.30	17.90	19.70	nd	nd	4
HG-365	6.15	6.15	20.50	11.20	21.40	22.40	20.60	7
Priya-151	6.61	6.61	9.28	8.00	18.10	29.90	7.75	7
Swati-55	6.52	6.52	nd	17.60	25.90	nd	nd	4
Jyoti-555	6.05	6.05	8.89	18.40	22.00	33.70	nd	6
Pusa-Selection I	6.40	10.80	nd	18.70	19.30	52.20	nd	5
Pusa-Navbhar	6.38	6.38	9.60	9.20	18.30	36.40	nd	6
PNB	6.01	6.01	22.80	16.90	22.30	27.90	28.60	7
Neelam-51	6.34	6.34	9.48	9.25	23.50	35.90	nd	6
Local-Selection	7.87	nd	10.70	12.40	19.70	43.20	32.80	6

nd: not detected.

Table 4
Total phenolic content (TPC) and total flavonoid content (TFC) in leaf tissue in different cultivars of cluster bean from different states in India and DPPH free radical scavenging activity (RC50) of different cultivars of guar.

S. No.	Cultivars of cluster bean	TPC (mg GAE/g)	TFC (mg QE/g)	RC50 (% inhibition)
1	RGC-936	79.23 ± 0.87	5.30 ± 0.25	32.50 ± 0.76
2	RGC-1002	82.73 ± 0.32	7.76 ± 0.35	60.66 ± 1.20
3	RGC-1003	82.00 ± 1.30	7.76 ± 0.59	60.96 ± 1.55
4	RGC-1031	204.67 ± 3.09	4.26 ± 0.52	22.50 ± 1.31
5	RGC-1017	69.90 ± 2.47	4.70 ± 0.27	57.16 ± 1.59
6	RGC-1066	181.2 ± 15.99	12.43 ± 0.64	88.79 ± 1.56
7	HG-365	97.66 ± 1.10	11.16 ± 0.66	57.06 ± 0.64
8	Priya-151	84.26 ± 1.45	8.03 ± 0.09	60.06 ± 1.18
9	Swati-55	95.46 ± 0.43	11.06 ± 0.38	41.40 ± 1.32
10	Jyoti-555	93.96 ± 0.37	10.66 ± 0.72	51.96 ± 0.89
11	Pusa-Selection 1	97.33 ± 0.39	8.23 ± 0.24	49.33 ± 0.67
12	Pusa-Navbhar	85.36 ± 1.94	9.66 ± 0.44	78.36 ± 0.68
13	PNB	80.03 ± 0.84	6.46 ± 0.32	71.46 ± 1.07
14	Neelam-51	78.40 ± 0.81	5.50 ± 0.27	45.86 ± 2.24
15	Local-Selection	60.03 ± 0.98	5.83 ± 0.18	7.06 ± 0.38

Each value is expressed as mean ± standard deviation (n = 3).
TPC: total phenol content (TPC) analyzed as gallic acid equivalent (GAE) mg/g of extract.
TFC: total flavonoid content analyzed as quercetin equivalent (QE) mg/g of extract.
RC50: amount required for the 50% reduction of DPPH after 30 min.

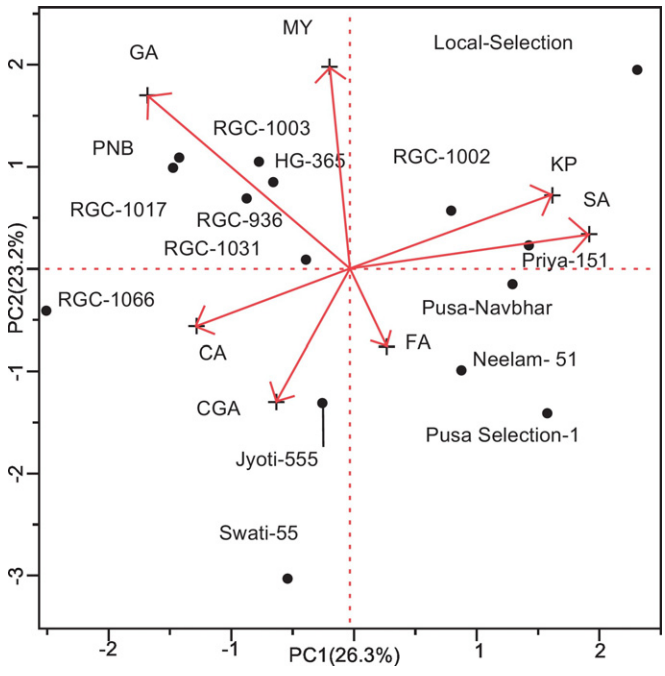


Fig. 2. Principle component analysis (PCA) of phenolic compounds in different guar cultivars. Parameter codes: myricetin MY, kaempferol KP, sinapic acid SA, ferulic acid FA, chlorogenic acid CGA, caffeic acid CA and gallic acid GA.

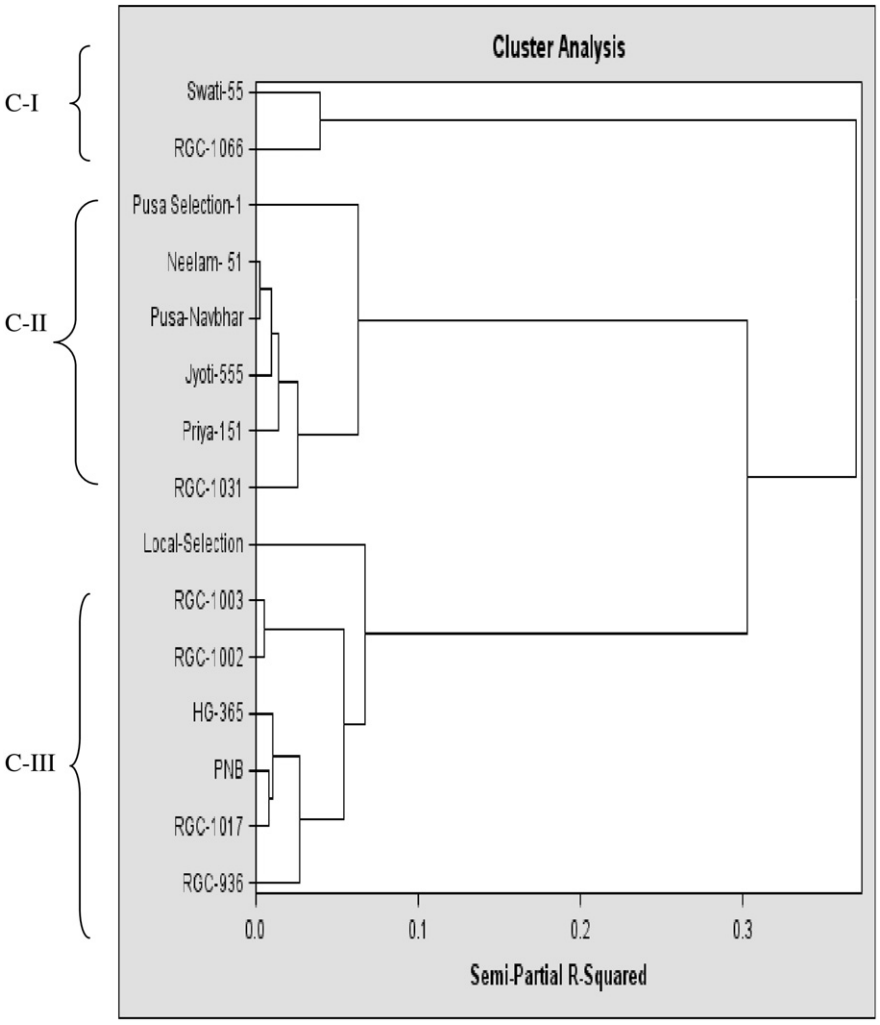


Fig. 3. Hierarchical clustering (HC) of guar cultivars on the basis of seven detected phenolic compounds.

indicates that at least two methods should be required for the comparison of the antioxidant activities and phenolic compounds [45].

Phenolics are known as radical scavengers or radical-chain breakers, extinguishing strongly the different oxidative free radicals. Extensive variability was observed in polyphenols contents among fifteen cultivars of guar. These cultivars showed linear correlation of antioxidant property with phenolic acids and flavonoids. In general, guar mainly contains low amounts of SA and a high amount of KP (Table 3). As an economic crop, guar not only contains a high amount of dietary fibre but also high amount of flavonoid KP (Table 3).

3.3. Multivariate analysis

The principal component analysis (PCA) and their correlation are shown in Fig. 2. The first principal component (PC1) represents 26.3% of variability, while the second principal component (PC2) represents 23.2% of variability among the data (Fig. 2). All phenolics compounds were occupied on both right and left side of the biplot and among the parameters the MY, GA, CA and CGA were observed on the left side of the bi-plot while KP and SA were observed on the right side of the biplot with high positive loading for both principal components. The KP and SA were found slightly together on the right middle side of the biplot suggesting a positive correlation with each other. Similarly, a positive correlation was observed between GA and CA.

Local-Selection and RGC-1002 showed high positive loading to both PC1 and PC2, while PNB, RGC-1017, RGC-1003, HG-365, RGC-936, RGC-1031 and RGC-1066 showed high positive loading to PC2. The results also showed that Local-Selection contained higher KP and MY while Pusa Selection-1 recorded the highest amount of KP. The cultivars RGC-936, PNB, Swati-55 showed relatively higher FA, GA and CGA, respectively (Fig. 2).

Hierarchical clustering (HC) analysis revealed that fifteen cultivars fall into three clusters having two major clusters (C-II and C-III) and one minor cluster (Fig. 3). The minor cluster I (C-I) includes two cultivars viz., Swati-55 and RGC-1066 showing fair amount of SA, FA, GA, CA, CGA while absence of KP and MY in both. The minor cluster II consists of Pusa-Navbhar, Pusa Selection-1, Neelam-51, Jyoti-555, Priya-151 and RGC-1031 having better amount of all phenolics along with much less amount or absence of MY. However, the RGC-1031 in this cluster showed higher amount of all analyzed phenolic acids except FA and MY. The cluster III consists of RGC-1002, RGC-1003, Local-Selection, HG-365, PNB, RGC-1017 and RGC-936 shows significantly higher amount of all the analyzed phenolic compounds (Fig. 3).

4. Conclusion

We have undertaken a thorough investigation in detection and quantification of the phenolic compounds in extracts from leaves of the medicinal plant guar i.e. *Cyamopsis tetragonoloba* (L.) Taub. Total phenolic contents in all cultivar were in the range 60.03 to 204.67 $\mu g \cdot gfw^{-1}$. It was also concluded that KP (52.20 $\mu g \cdot gfw^{-1}$) is the major constituent in the studied guar cultivars in comparison to

other phenolics such as SA, CA, GA, FA, CGA and MY. Our results show the extensive variability in phenolic compounds among fifteen cultivars of guar. On the basis of PCA and cluster analysis of the cultivars it was concluded that Jyoti-555 of Gujrat is significantly similar to RGC-1031 of Rajasthan. These cultivars could be exploited as sources of natural antioxidants. However, further research is essential to evaluate the biological activity of these phenolics in assessing the possible desirable effect improving consumer's health.

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