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Characterization of scented geranium accessions for odour and chemical compositions in southern transitional zone of Karnataka

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ABSTRACT : Scented geranium (*Pelargonium* spp.: Geraniaceae) is an important, high value aromatic crop of South African origin. Due to high demand and price for the oil, an excellent potential exists for increasing cultivated area in India. An attempt was made to evaluate and characterize the available accessions of scented geranium based on their physico-chemical properties, chemical composition and odour assessment of the oil. There were seven treatments and four replications. All the accessions possessed light yellow coloured oil except PG-10, which possessed light green colour. The oil from PG-10 showed maximum acid value (3.02). The oil from PG-8 recorded the highest ester value (58.737). The oil form KB and CIMAP possessed maximum citronellol and geraniol content while, linalool was found to be maximum in case of PG-11. PG-10 contained maximum isomanthone (8.50 %). The oil from PG-1 recorded the highest citronellyl formate content (15.83%). The essential oil was distilled during August from seven accessions upon GC analysis. The concentrations of isomenthone, citronellyl formate and citronellol were maximum in case of PF-10 (7.74, 23.18 and 37.07%, respectively). The major alcohols *i.e.*, citronellol, geraniol, linalool and nerol were maximum in case of the oils of KB, CIMAP, PG-8 and PG-1 during May. Whereas, during August, PG-10 recorded the maximum nerol and cironellol whereas, PG-12 registered the highest concentration of geraniol and linalool. The rosy odour of different accessions is attributed to the presence of higher levels of geraniol, which is evident in the present case with the accession PG-12, during August. Hence, all the accessions differed significantly in all the parameters.

KEY WORDS : Geranium, Accessions, Physico-chemical properties, Chemical composition, Odour assessment

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India's biodiversity coupled with its vast resources including competitive workforce, highly intelligent scientific and rich business community make our country the best choice for growing aromatic crops for world market. The field of aromatic crops is assuming importance because of growing demand for natural flavours throughout the world. Many exotic and indigenous essential oils are being utilized by fragrances are industries to create high grade perfumes.

Scented geranium (*Pelargonium* spp. : Geraniaceae) is an important, high value perennial aromatic crop of South African origin, that was introduced into India during 1900-1915 AD by French planters and got acclimatized to South Indian climate.

The oil has a fine rosy odour and a rich long lasting sweet rosy dry out note (Sastry *et al.*, 2000). More than 120 constituents were identified in the oil, the major ones being citronellol, geraniol, linalool, isomenthone, citronellyl formate, citronellyl acetate, geranyl formate, geranyl acetate, guia 6, 9-diene, 10-epi-γ- eudesmol etc. due to high demand and price for the oil, an excellent potential exists for increasing cultivated area in India (Rao, 2000).

Presently, the classification of geranium is based on morphology and/or chemical composition of the oil. The morphological characteristics and physico-chemical properties of the oil are key issues in characterizing the genotypes / accessions, but most of the times these characters are highly influenced by environmental conditions. Hence, the data obtained by such evolutions are not easily understood at generic level, often resulting in maintenance of duplicate accessions/genotypes. So, characterizing the generic diversity existing in various genotypes/ accessions of a species is of great importance especially to plant breeders.

An attempt was made to evaluate and characterize the available accessions of scented geranium based on their physico-chemical properties, chemical composition and odour assessment of the oil and molecular diversity.

RESEARCH METHODS

For morphological evaluation, the experiment was conducted at the Division of Horticulture, Ghandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore.

This experiment was conducted using seven geranium accessions, which are given in Tables A and B.

The land was ploughed once with mould board plough and harrowed twice. The treatments were allotted to the experimental plots by using random Table A. Shoot tip cuttings of 15-20 cm long with 4-5 nodes together

Table A: Geranium accessions

Sr. No.	Accession (Treatment)	Source
1.	PG-1	IIHR
2.	PG-8	IIHR
3.	PG-10	IIHR
4.	PG-11	IIHR
5.	PG-12	IIHR
6.	CIMAP Collection	GKVK-CIMAP
7.	Kodiakanal Bourbon	IIHR-HRS, KODIAKANAL

Table B: Design and layout

Treatments	:	Seven
Replications	:	Four
Plot Size	:	3 M x 2.4 M
Fertilizers	:	120:40:40 kg NPK/ha
Spacing	:	60 cm x 60 cm

with terminal bud crown of leaves were selected from healthy field grown plants. The cuttings were given a prophylactic treatment of bavistin solution (0.02%). Later, cut ends of the cuttings were dipped in IBA (1500 ppm) solution for 30 seconds. The cuttings were planted in polythene bags containing rooting mixture (Sand: FYM - 3:1) for rooting and arranged in mist chamber. After 45 days rooted cuttings were shifted outside for hardening. The hardened rooted cuttings were shifted to GKVK and transplanted to the experimental plots.

The plots were levelled. Ridges and furrows were opened at 60cm apart. Then the rooted cuttings were planted on one side of the ridge and irrigated immediately after planting.

Farmyard manure at the rate of 10 kg/plot was applied one month before planting. FYM was spread uniform in each plot and mixed thoroughly with the soil. Nitrogen in the form of urea (46 % N), phosphorus in the form of single phosphate (16 % P_2O_5) and potassium in the farm of muriate of potash (60 % K_2O) were supplied at the rate of 120:40:40 kg per hectare. $1/4^{th}$ of nitrogen and entire dose of phosphorus and potassium were applied as a basal dose. Remaining dose of nitrogen was applied in three equal splits after every harvest and between one and half months after harvest.

The crop was harvested when the leaves turned to light green colour from dark and when they started blooming, as the oil content is reported to be maximum during that time. Plants were cut at 15-18 cm from the apex to ensure good regeneration capacity, using secateuer to avoid any shock damage to the plants. The first harvest was at flowering, which indicated the maturity of crop. The second harvest was done during August that is three months after the first harvest, which acted as a pruning process, which helped faster regeneration of new shoots.

The following methods were employed:

– Clevenger apparatus (hydro distillation)

– Mini pilot scale distillation (steam distillation) unit

Both these methods were employed to know the oil content and yield. About 100g of the fresh herbage of each accession was distilled in Clevengers apparatus for all the four replications. The period of distillation was kept constant for $3_{1/2}$ hours. The mini pilot scale steam distillation the herb and here also the period of distillation was kept constant for $3_{1/2}$ hours.

Physico-chemical properties of the oil:

The quality of the oil was judged based on their physico-chemical properties, which are mentioned below.

Physical parameters :

Colour:

The colour of the essential oil of seven geranium accessions was recorded through visual means.

Specific gravity :

Specific gravity of the oils of the accessions was measured at room temperature by using the following formula:

$$d \frac{RT}{RT} = \frac{\text{Weight of 10 ml sample}}{\text{Weight of 10 ml water}}$$

where,

d RT = RT is the specific gravity at room temperature.

Density:

Specific of oil was calculated based on mass by volume basis at room temperature and expressed in gram per cubic centimeter.

Refractive index [n_D^{RT}]:

Refractive index was measured using a refractrometer at room temperature.

$$n_D^{RT} = \frac{\text{Since of angle of incidence}}{\text{Since of angle of refraction}}$$

where 'n_D^{RT}' is the refractive index of the oil at room temperature.

Optical rotation [$\sim_D^{RT}$]:

The optical rotation of the oil was measured by using a polarimeter and expressed in degrees of rotation.

Chemical properties:

Acid value (*AV*) :

Acid value is the number of milligrams of sodium hydroxide required to neutralize one gram of oil. The oil was dissolved in an appropriate solvent (methanol), later the solution is titrated with 0.1 N NaOH solution. The amount of NaOH solution consumed gave a measure of acidity of the oil, by the following formula.

$$AV = \frac{56.1NV}{W}$$

where,

AV – Acid value

N – Normality of NaOH

V – Volume (ml) of NaOH

W – Weight of the sample (oil).

Saponification value (*SV*) :

Saponification value denotes the number of milligrams of sodium hydroxide required to saponify one gram of oil. SV was determined by completely saponifying the oil with a known amount of sodium hydroxide, the excess of which determined by titration with a standard acid.

$$SV = \frac{56.1NV(V_2 - V_1)}{W}$$

where,

SV – Saponification

N – Normally of NaOH

V_2 – Volume of NaOH used blank

W – Weight of the oil sample.

HV is a measure of the free hydroxyl groups present in known weight of the sample (oil).

Gas chromatographic analysis of the oil of different accessions:

The oil samples were filtered through magnesium sulfite to remove the traces of moisture and their pH

Table C : Equipment and analytical conditions

1.	G.C. Model	:	Varian 3700 series
2.	Column	:	Stainless steel 3 M x 2 mm id (internal diameter) Column packed with carbowax 3M on chromosorb 80/100 WHP
3.	Carrier gas	:	UHP nitrogen
	Flow chart	:	30 ml/min
	Pressure	:	1.2 kg/cm^2
4.	Detector	:	FID Flame ionization detector) 0-1000 mv
	H_2 pressure	:	1.2 kg/cm^2
	Air pressure	:	2 kg/cm^2
	Flow chart	:	30 ml/min (for both air and H_2)
5.	Temperature parameters	:	
	Column temperature programme	:	100…… → 220°C (60 min)
	Ramping rate	:	2° C/min
	Injector temperature	:	250°C
	Detector temperature	:	280°C

was checked before performing the test (Because trace amounts of moisture present in the sample and/or acidic condition, if prevails, destroys the GC column). Only a very little amount (1-2 µ1) of the oil was injected into the injector port of the GC using microsyringe. The details of the equipment and the analytical conditions are described Table C.

Finally, the essential oil components in samples were identified by their peak retention times those of authentic standards run under identical conditions. The GC was performed for both hydro-distilled and steam distilled oils.

Odour evaluation:

One month old oil samples of first harvest were evaluated for odour characteristics by a professional perfumer. All the three notes *i.e.*, top note, body (or middle or heart) note and base (or after or dry out or bottom) note were taken onto account while assessing the odour characteristics of the oil from each the accession.

Statistical analysis and interpretation of data:

The data growth and yield parameters were subjected to Fisher's method of analysis of variance as outlined by Sundararaj *et al.* (1972). Wherever the 'F' test was significant for comparison of treatment means, critical difference (C.D) values were calculated at 5 per cent probability level.

RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Physical parameters:

Generally all the accessions possessed light yellow coloured oil except PG-10, which possessed light green colour. The intensity of yellow colour varied among the accessions. Differences in the colour of the essential oil may be attributed to the presence of colouring pigments at different proportions. The light green colour of the oil from PG-10 could be due to more of chlorophyll whereas, in other, carotenoids might have been at higher proportions. Anonymous (1988) reported a yellowish brown coloured geranium oil.

The BIS (Bureau of Indian Standards) specifications for geranium oil were reported (Anonymous, 1988). The colour of the oil should be yellow to yellowish brown with a density ranging between 0.8824 to 0.8966, refractive index should fall within 1.4633 to 1.4728 and optical rotation to be in the range of -7° to - 11°. However, the present findings indicated that the physical parameters of the oil were within the specifications of BIS (Except for optical rotation, the oil from PG-1, PG-8 and CIMAP showed slightly higher optical rotation values than the BIS specification).

Chemical properties :

Acid value :

The oil from the accessions PG-10 showed maximum acid value (3.02), while it was minimum in case of KB (2.14) as against a maximum acid value of 10 as specified by BIS (Anonymous, 1988). These results could be due to the presence of higher free acid content in PG-10 and lower free acid content in Ranade (1998) and Kumar *et al.* (1985) ranged from 4.10, 5-10 and 1.5-3.22, respectively for different geranium oils.

Easter value :

Among the different accessions, the essential oil from PG-8 recorded the highest ester value (58.737), which could be due to the higher percentage of citronellyl acetate and other esters present in that oil. However, the oil from PG-12 recorded the lowest ester value (40.360) and it could be because of lower percentage of neryl formate, citronellyl formate and other esters. In

Table 1 : Physico-chemical properties of scented geranium oil from different accessions physical parameters

Sr. No.	Accessions	Colour	Specific gravity (at RT)	Density	Refractive index	Optical rotation	Acid value	Ester value	Hydroxyl value (OH)
1.	PG-1	Light yellow	0.88846	0.9035	1.4648	-12°48'	2.77	41.790	206.829
2.	PG-8	Light yellow	0.8906	0.9115	1.4666	-11°36'	2.41	58.737	286.821
3.	PG-10	Light yellow	0.8834	0.9041	1.4584	-9°30'	3.02	46.000	211.937
4.	PG-11	Light yellow	0.8918	0.9108	1.4657	-10°	2.72	40.990	210.197
5.	PG-12	Light yellow	0.8931	0.9122	1.4668	-10°42'	2.96	40.360	226.837
6.	KB	Light yellow	0.8842	0.9077	1.4658	-11°	2.14	47.700	219.546
7.	CIMAP	Light yellow	0.8887	0.9031	1.4649	-11°18'	2.76	45.600	290.628

general, the ester values for different accessions varied widely. Ranade (1998) found, the ester value to range from 31-78 for different geranium oils. Kumar *et al.* (1985) found ester values ranging from 50-75 for two types of geranium oils (Reunion oil and Nilgir oil). Their observation supports the present findings for the variation in ester value among the oils of seven geranium accessions.

Hydroxyl value :

The essential oils of different geranium accessions varied considerable for the hydroxyl value, which ranged from 206.829 to 290.628 in case of PG-1 and CIMAP, respectively. Primarily the variation in hydroxyl value is due to the differences in the proportion of major alcohols such as linalool, citronellol, geraniol and nerol among the accessions. The lowest hydroxyl value recorded in PG-1 is well reflected in its lower citronellol content (18.522% - Table 1). The highest hydroxyl value of CIMAP is due to the fact that the oil from CIMAP accession possessed higher amounts of geraniol (26.285%). The variation in the hydroxyl value among the accessions is largely due to the differences in their free hydroxyl groups present in their oils. CIMAP oil possessed higher total alcohol content (61.253 and 64.476 per cent in hydro and steam distilled oils during May). However, the steam-distilled oil (May) from the accession PG-8 recorded the highest total alcohol content (66.311%).

Gas chromatographic analysis of the essential oil :

The data on chemical composition of essential oil of geranium accessions are given in Table 2 and 3. Ten components of the oil were identified of which six major and the results indicated that considerable variation exists in the proportion of the major components in the oils among the accessions and within the accessions, the composition also varied depending on the harvest period

Table 2 : Principal components (%) of the steam distilled oils of scented geranium accessions

Sr.	Accessions	PG-1		PG-8		PG-10		PG-11		PG-12		KB		CIMAP	
No.	Components	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1.	Isomenthone	7.717	5.708	7.240	5.324	8.500	7.739	6.560	5.081	7.398	4.724	6.163	5.802	6.439	5.913
2.	Linalool	8.644	7.371	8.614	6.546	3.675	2.413	9.787	8.349	8.583	8.703	6.952	7.863	7.573	7.003
3.	Citronellyl formate	15.534	11.903	15.129	12.377	15.236	23.183	13.818	10.716	12.104	11.516	11.222	13.583	7.276	12.290
4.	Citronellyl acetate	13.831	12.251	12.248	13.075	3.361	4.925	14.073	13.154	10.751	13.19	10.315	12.348	4.059	12.290
5.	Citronellol	18.522	22.314	17.352	21.341	23.529	37.073	21.874	17.674	18.467	21.246	31.026	23.061	26.859	22.662
6.	Geraniol	15.743	19.957	14.536	19.461	5.095	4.362	13.748	20.505	15.495	17.587	20.559	20.393	26.285	19.662
7.	Neryl formate	0.045	0.096	0.117	0.366	3.361	0.751	0.106	0.365	0.231	0.299	0.079	0.287	T	T
8.	Geranyl formate	T	0.955	0.729	0.189	4.908	0.726	T	0.475	3.691	0.717	0.220	0.886	0.936	0.361
9.	Nerol	0.877	0.261	0.261	0.390	4.733	2.651	2.361	0.263	1.838	0.253	0.440	0.145	0.563	0.142
10.	Geranyl acetate	0.944	0.128	0.730	0.935	2.362	1.373	0.905	0.692	0.865	0.452	T	0.496	2.353	1.096

A: The oil distilled during May, B: The oil distilled during Aug, T: Traces

Table 3: Principal components (%) of Clevenger (Hydro) distilled oils of scented geranium accessions

Sr.	Accessions	PG-1		PG-8		PG-10		PG-11		PG-12		KB		CIMAP	
No.	Components	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1.	Isomenthone	8.488	6.085	6.996	4.128	10.316	10.449	7.072	5.851	8.057	5.799	6.682	6.585	6.903	5.919
2.	Linalool	10.119	10.105	15.488	4.698	3.002	0.963	7.248	8.188	9.854	10.768	5.021	6.626	8.071	7.508
3.	Citronellyl formate	10.978	9.865	6.884	7.873	15.052	21.413	8.824	6.509	8.668	6.476	7.3188	9.081	7.592	9.391
4.	Citronellyl acetate	5.985	9.961	1.794	9.561	2.702	2.418	4.409	7.421	4.929	9.069	4.096	9.287	2.750	9.489
5.	Citronellol	18.753	21.516	25.210	19.998	29.534	42.896	20.246	18.915	19.666	18.508	29.900	19.191	27.725	19.432
6.	Geraniol	17.320	20.016	24.940	30.354	6.705	2.847	17.948	26.336	16.720	30.205	21.652	26.158	28.121	24.901
7.	Neryl formate	0.077	0.152	T	0.131	0.022	0.067	T	0.436	0.105	T	T	0.182	T	0.185
8.	Geranyl formate	1.195	0.121	1.199	0.178	0.945	0.469	1.480	0.463	1.054	0.347	0.955	0.222	2.750	0.170
9.	Nerol	3.040	0.871	0.673	0.765	2.619	1.734	1.316	0.877	1.047	0.740	0.474	0.694	0.559	0.709
10.	Geranyl acetate	3.174	0.213	1.794	0.847	2.703	2.418	3.413	0.826	3.550	0.445	2.581	0.802	0.775	1.083

and the method of distillation.

Composition of the essential oil obtained through steam distillation :

The essential oil distilled during May from the seven accessions upon GC analysis possessed the following composition, which varied among the accessions (Table 2). The oil from the accession PG-10 contained maximum isomanthone (8.50%), whereas, KB possessed the lowest isomenthone content (6.16%). Linalool was highest in case of the accession PG-11 (9.79%) and lowest PG-10 (3.68%). The oil from accession PG-1 recorded the highest citronellyl formate content (15.83%), which, it was least in case of CIMAP (7.28%). Further, citronellyl acetate was found to be maximum in PG-11 (14.07%) and lowest in PG-10 (3.36%). Citronellol (Once of the major alcohols) was found to be highest in case of KB (31.03%) and lowest in PG-8 (17.35%), while geraniol (another major alcohol) was highest in case of CIMAP (26.29%) and lowest in PG-10 (5.10%).

The essential oil distilled during August from seven accessions upon GC analysis recorded the following composition (Table 2).The concentrations of isomenthone, citronellyl formate and citronellol were maximum in case of PF-10 (7.74, 23.18 and 37.07%, respectively). Whereas, PG-12 recorded the lowest isomenthone (4.72%), PG-11, the lowest citronellyl formate (10.72%) and citronellol (17.67%). Linalool was found to be highest in case of PG-12 (8.70%) and lowest in PG-10, (2.41%). PG-12 recorded the maximum citronellyl acetate (13.19%) while, PG-10, the lowest (4.93%). Geraniol was found to be maximum in PG-11 (20.51%) and minimum in PG-10 (4.36%).

Composition of the essential oil obtained through Clevengers apparatus (Table 3) :

The major alcohols *i.e.*, citronellol, geraniol, linalool and nerol were maximum in case of the oils of KB, CIMAP, PG-8 and PG-1 during May month. Whereas, during August month, PG-10 recorded the maximum nerol and cironellol whereas, PG-12 registered the highest concentration of geraniol and linalool.

If we compare the two distillation methods, it is clear that the concentration of alcohols (especially citronellol, geraniol and linalool) is maximum in the oils, which are hydro distilled. These results are in conformity with the findings of Kaul *et al.* (1995). It is also known that, the recovery of esters is generally more in case of steam distilled oils that hydrodistilled oils confirming the results obtained in this study.

It has been reported that during steam distillation many of the trace compounds like cis-3-Hexanol, 2-trans 6-cis – nonadian-1-ol etc., which are responsible for odour are lost due to their solubility in water. The pH of the water also plays a very important role in hydro distillation. The active molecules like L-citronellol, isomenthone, menthone, linalool, rose oxide, citronellyl formate and 10-epi-γ-eudesmol are subjected to change in the ratio based on the pH of water, which results in substantial changes in the ratio based on the pH of water, which results in substantial changes in overall odour pattern of the oil (Harlalka, 2000).

The odour characteristics (Table 4) differed considerably among the oils obtained from different accessions. This is largely due to the differences in their chemical composition. The rosy odour of different accessions is attributed to the presence of higher levels of geraniol (rose alcohol), which is evident in the present case with the accession PG-12, its oil recorded maximum geraniol (30.205%) during August (Hydro distilled oil).

The harshness of the oil of PG-10 could be very well related to its geraniol content, which is lowest among all the accessions at both the harvest and in two distillation methods. Accession CIMAP has got a more balanced

Table 4: Odour characteristics of the essential oil of geranium accessions

Sr. No.	Accessions	Odour		
		Top note	Middle [or Body] note	Dry out [Bottom] note
1.	PG-1	Rosy / minty	Rosy	More rosy
2.	PG-8	Less rosy	Rosy	Strong / rosy
3.	PG-10	Very harsh / pungent	Less harsh	Very less harsh / ordinary
4.	PG-11	Rosy / oily side note	Rosy / less strength	No change
5.	PG-12	Rosy	Balanced	Strong / tenacious / stable
6.	KB	Balanced	More rosy	Rounded
7.	CIMAP	Greenish / Tagetes like	Rosy with more formates	Rosy with tagetes smell

and rounded odour which could be due to the fact that, the oil from this accession possessed citronellol and geraniol in almost 1:1 proportion. The oil from KB possessed a greenish top note with tagetes like smell and this could be due to the presence of higher amounts of formates and other minor constituents.

The oil from PG-1 upon odour evaluation confirmed to have a good odour with a better blending property with the other essential oils, whereas, the odour value of PG-8 revealed that it possesses a powerful nice base odour. Both PG-10 and PG-11 seems to be of poorer odour value because of their harshness and oily side note. PG-12 possessed a unique odour and found to have better perfumery value, whereas the oil from KB to be of good quality.

The essential oil of CIMAP possessed a rounded and more balanced odour and has got a better chance to be preferred by the perfumery industry due to its balanced proportions of citronellal and geraniol.

In the order of preferences, CIMAP stands first followed by PG-8, PG-12, KB and PG-1 which will be of better odour value whereas, PG-10 and PG-11 were not at all good for perfumery because of their harshness and oily side note. However, the blends of PG-1+PG-12, PG-8+PG-12 and PG-1+PG-8 will be a better prospect for perfumery industry as revealed by the odour evaluation expert.

Though the differences in chemical composition lead to different odour, it is the origin, which ultimately has a major role to play in deciding the odour as reported by Anonymous (2001) in case of rosemary and Demarne (1989) in scented geranium.

Conclusion:

All the accessions differed significantly in all the phsico-chemical parameters. Steam distilled oil was found better than hydro-distilled oil based on their principal components confirming the results obtained in this study.The odour characteristics differed considerably among the oils obtained from different accessions.In the order of preferences, CIMAP stands first followed by PG-8, PG-12, KB and PG-1 which will be of better odour value whereas, PG-10 and PG-11 were not at all good for perfumery because of their harshness and oily side note.

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