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Extraction of essential oil in different accessions of *Calendula officinalis* Linn.

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Abstract

Calendula officinalis L. is globally known for its medicinal importance. It has an extremely wide range of use, due to its certain properties and is well adapted to temperate climatic regions and is easy to grow. In present study, 12 accessions of *Calendula officinalis* viz., *Calendula officinalis* Touch of Red Buff, *Calendula officinalis* Golden Emperor, *Calendula officinalis* Fiesta Gitana Mix, *Calendula officinalis* Creamy White, *Calendula officinalis* Balls Orange, *Calendula officinalis* Balls Yellow, *Calendula officinalis* Fiesta Gitana Orange, *Calendula officinalis* SKUAST-K Source, *Calendula officinalis* Kashmir University Source, *Calendula officinalis* Floriculture department Source (Emporium garden), *Calendula officinalis* Agriculture department Source (Lal mandi), *Calendula officinalis* Local variety (Source: nursery) were analyzed for their growth parameters and oil content. The trial was laid using randomized complete block design (RCBD) and the data was analyzed in R Studio, version 4.2.2, 2022 to determine the best accession in temperate climate of Kashmir. The growth parameters like no. of flowers/plant (17.11), Weight of fresh flowers/p (41.73 g), Weight of dry flowers/p (9.78 g) was recorded highest in Lal-mandi. Highest oil content (0.472%) was recorded in Fiesta gitana orange and lowest in the creamy white (0.069%). All these parameters were recorded when the plants were in full bloom.

Keywords: *Calendula officinalis*, medicinal, accessions, temperate

Introduction

The genus *Calendula* (Asteraceae) includes round about 25 herbaceous annual or perennial species, most common being *Calendula officinalis* Linn., *Calendula arvensis* Linn., *Calendula suffruticosa* Vahl., *Calendula stellata* Cav., *Calendula alata* Rech., *Calendula tripterocarpa* Rupr. The genus is native to the Mediterranean countries (Arora *et al.* 2013) [1]. *Calendula officinalis* L. (English marigold, pot marigold) belonging to the Asteraceae (Compositae) family is an annual herbaceous plant (Rigane *et al.* 2013) [12]. It is a popular medicinal plant in India, China, Europe, and the United States. Because of the plant's extended blossoming cycle, its name originates from the Latin word 'Calend,' which means the first day of each month. *Calendula* has corymbosely branched stem, a long tap root with several subsidiary roots, hispid, acute, oblanceolate, alternating, and sessile leaves, and a flower head inflorescence that grows to about 80 cm tall surrounded by two rows of hairy bracts. Flowers range in colour from yellow to orange, with female ray florets and hermaphrodite, tridentate, tubular disc florets; and curved, sickle-shaped, and ringed achenes (Jan *et al.* 2017) [5].

The extracts of pot marigold possess a wide range of pharmacological effects (Pintea *et al.* 2003) [9]. It has traditionally been used to treat internal organ inflammations, gastrointestinal ulcers, and dysmenorrhea, as well as a diuretic and diaphoretic in convulsions. It is also used to treat oral and pharyngeal mucosa inflammations, as well as cuts and burns. *Calendula* is a cleaning and cleansing herb, and its infusion is used to treat persistent illnesses. In wounds, markings, freckles, sprains, and conjunctivitis, a topical application of floral infusion is utilized as an antifungal and antimicrobial. *Calendula* tea is used for eyewashes, gargles, diaper rashes, and various skin and mucous membrane inflammatory diseases. *C. officinalis* mother tincture is used in homoeopathy to relieve mental strain and insomnia (Arora *et al.* 2013) [1].

The leaves and blossoms of *C. officinalis* contain antipyretic, anti-inflammatory, antiepileptic, and antibacterial effects, according to the Ayurvedic and Unani systems of medicine. *C. officinalis* has been used for impaired eyesight, menstrual irregularities, varicose veins, haemorrhoids, and duodenal ulcers in both traditional and homeopathic treatment. Calendula flowers were used in the Middle Ages to treat liver blockages, snake bites, and to strengthen the heart. In the 18th century, it was used to treat headaches, jaundice, and red eyes. During the Civil War, the plant was used to cure wounds and as a treatment for measles, smallpox, and jaundice (Arora *et al.* 2013)^[1].

Phytochemicals found in the *Calendula officinalis* L. include carbohydrates, phenolic compounds, lipids, steroids, tocopherols, terpenoids, quinones, and carotenoids, all of which have various health effects. Triterpenoid esters, saponins, and flavonoids such as rutin and hyperoside are among the plant's most active ingredients. Carotenoids such as auroxanthin and flavoxanthin are abundant in the orange bloom (Jan *et al.* 2017)^[5].

The fragrance of plants is carried in the so called quinta essential, or essential oil fraction (Bilal *et al.* 2022)^[2]. *Calendula officinalis* essential oils have a number of therapeutic properties, including anti-inflammatory, anti-tumorogenic, and cicatrizing effects. Furthermore, its oil's *in vitro* antibacterial activity have been proven. It has been shown that plant extracts exhibit action against HIV-1 replication. The essential oils of *C. officinalis* have also been shown to have a genotoxic impact, and the plant is known to have wound-healing and antioxidant qualities (Okoh *et al.* 2008)^[8].

In this study we report the percentage of essential oil extracted from 12 different accessions of *Calendula officinalis* flowers.

Materials and Methods

The present experiment was carried out at in Nursery of Division of Forest Products and Utilization, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal Kashmir, which is located within the university campus at an altitude of 1757 m above mean sea level, between 34° 16' N latitude and 74° 46' E longitude. The experimental site falls in a mid to high altitude characterized by the mid summers and severe winters. The average annual rainfall at the site is 820 mm per annum, most of which is received during rainy and winter season. The site also experiences an average snowfall of about 2–3 ft during winter. The mean meteorological data for the experimentation period obtained from the meteorological observatory Ganderbal. The experimental site receives a maximum sunshine hours of 12.30 per day. The mean maximum relative humidity of the site is about 82.45% and mean minimum relative humidity is 54.49% per annum and mean maximum temperature is 19.6° C and mean minimum temperature is 6.8° C per annum. The soil of the study site is sandy loam in texture; high in organic carbon with slightly neutral in pH and normal in electrical conductivity.

The experiment was laid at a spacing of 25 x 25 cm with 12 treatments (accessions) of *Calendula officinalis* viz.,

Calendula officinalis Touch of Red Buff, *Calendula officinalis* Golden Emperror, *Calendula officinalis* Fiesta Gitana Mix, *Calendula officinalis* Creamy White, *Calendula officinalis* Balls Orange, *Calendula officinalis* Balls Yellow, *Calendula officinalis* Fiesta Gitana Orange, *Calendula officinalis* SKUAST-K Source, *Calendula officinalis* Kashmir University Source, *Calendula officinalis* Floriculture department Source (Emporium garden), *Calendula officinalis* Agriculture department Source (Lal mandi), *Calendula officinalis* Local variety (Source: nursery) and 3 replications through randomized complete block design (RCBD), accordingly the data was analyzed in R Studio, version 4.2.2, 2022 (R Development Core Team, 2022)^[10].

The study was carried out for 2 years. Some accessions of *Calendula officinalis* were collected from different departments of Kashmir region while others were brought from Punjab Agricultural University in 2021 and were sown in trays in the mid December inside polyhouse. The seedlings were transplanted in field after 3 leaf stage. To sustain their rapid growth, the plants received irrigations all during the growing season. Weeding was done by hand once a month. The data for observations were recorded when the flowers were in full bloom. Dried flowers were taken in Clevenger-type apparatus for oil extraction. The essential oil was isolated by hydro distillation method and hydro distillation was carried out for 4 h for isolation of essential oil.

Results

The parameters which were recorded were No. of flowers /plant, Weight of fresh flower/plant (g), Weight of dry flower/plant (g), Total oil content (%). The data in Table 1 illustrates that maximum no. of flowers/ plant were recorded in Lal-mandi source (17.11) and the least no. of flowers were recorded in Golden emperor (2.55).

Table 1: No. of flowers/plant in different accessions of *Calendula officinalis*

Treatments	No. of flowers/plant
Lal-mandi	17.11 (1.23)
Nursery	14.55 (1.16)
KU	12.77 (1.10)
Emporium garden	11.22 (1.04)
SKUAST-K	8.88 (0.97)
Fiesta gitana orange	8.22 (0.94)
Fiesta gitana mix	7.77 (0.91)
Touch of red buff	6.00 (0.81)
Balls orange	5.22 (0.75)
Balls yellow	4.33 (0.68)
Creamy white	3.66 (0.61)
Golden emperor	2.55 (0.48)
CD ($p \leq 0.05$)	0.01
W ($p \leq 0.05$)	0.31

* The values within parenthesis are log transformed values

Weight of fresh flowers/p (g) and Weight of dry flowers/p (g) was recorded highest in Lal-mandi source (41.73) and (9.78) respectively whereas the least weight of fresh flowers/p (g) and weight of dry flowers/p (g) was recorded in Golden emperor (6.37) and (1.43) respectively (Table 2).

Table 2: Fresh flower weight/plant and dry flower weight/plant in different accessions of *Calendula officinalis*

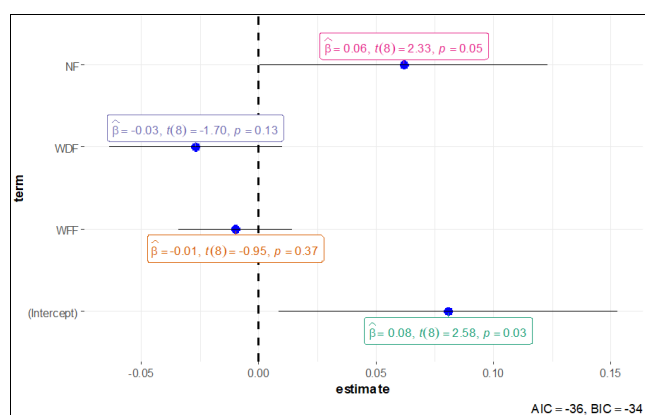
Treatments	Weight of fresh flowers/p (g)	Weight of dry flowers/p (g)
Lal-mandi	41.73 (1.62)	9.78
Nursery	33.48 (1.52)	6.24
KU	30.59 (1.48)	4.58
Fiesta gitana orange	26.54 (1.42)	3.98
Emporium garden	21.29 (1.32)	3.23
Fiesta gitana mix	21.23 (1.32)	3.18
SKUAST-K	16.55 (1.21)	3.01
Creamy white	14.08 (1.14)	2.46
Touch of red buff	9.53 (1.00)	2.11
Balls orange	8.32 (0.94)	2.00
Balls yellow	8.04 (0.93)	1.53
Golden emperor	6.37 (0.83)	1.43
CD ($p \leq 0.05$)	0.04	0.26
W ($p \leq 0.05$)	0.122	4.49

* The values within parenthesis are log transformed values

The data in TABLE 3 revealed that the highest percentage of the oil was obtained from Fiesta gitana orange (0.472%) and the least was obtained from Creamy white (0.069%).

Table 3: Total oil content (%) in different accessions of *Calendula officinalis*

Treatments	Total oil content (%)
Fiesta gitana orange	0.472
Balls orange	0.429
Touch of red buff	0.417
Fiesta gitana mix	0.400
Lal-mandi	0.355
Emporium garden	0.329
KU	0.318
SKUAST-K	0.295
Nursery	0.282
Balls yellow	0.227
Golden emperor	0.149
Creamy white	0.069
CD ($p \leq 0.05$)	0.010
W ($p \leq 0.05$)	0.002

**Fig 1:** The results were also inferred through regression model

Discussion

The number of inflorescences is a vital feature differentiating the varieties of *Calendula officinalis*. Król, 2012 [7] reported in his study that no. of flowers produced varied from one cultivar to another. Crnobarac *et al.*, 2009 [3] reported in his experiment that pot marigold produced about 30 flower heads whereas Khalid *et al.*, 2006 [6] reported that this number varied from 70 to 140 per plant. Such big difference of this value results from the differences in experimental factors and climatic conditions. Results of

the current study go in parallelism with these previous studies.

In our study weight of fresh flowers/p (g) and Weight of dry flowers/p (g) was recorded highest in Lal-mandi source whereas the least weight of fresh flowers/p (g) and weight of dry flowers/p (g) was recorded in Golden emperor. The reason for this could be correlated with the no. of flowers /plant which was highest in Lal-mandi and lowest in Golden emperor. Some varieties like Creamy white had big flowers due to which it had more weight of flowers/plant when compared to Balls orange and Balls yellow. The yield of *Calendula officinalis* raw material (flower heads) depends mainly on climatical conditions and the cultivar characteristic. The yield of dried flowers ranged from 1000 to 2000 kg/ha (Król, 2012) [7] which is in support of our findings. Similar results were obtained by Crnobarac *et al.*, 2009 [3].

The current study demonstrated that highest percentage of the oil was obtained from Fiesta gitana orange and the least was obtained from Creamy white on distillation. This could probably be due to the difference in their colour where Fiesta gitana orange flowers have deep orange colour and Creamy white have fade yellow colour. Similar results were found by Król, 2012 [7] where he recorded maximum essential oil content in orange flowered cultivars and least in yellow ones with the exception only in case of cultivar 'Orange King', which had significantly less essential oil in comparison with the cultivar 'Santana' with yellow flowers. Raal *et al.*, 2016 [11] recorded maximum oil percentage of 0.43% in double ball cultivar which had orange coloured inflorescence. Gazim *et al.*, 2008 [4] in their study which was carried out in Brazil revealed that the oil percentage obtained from dried flowers was 0.1%. Orange cultivars of calendula yield higher oil content and it may occur that naturally occurring higher oil concentrations coexist with pigments linked to orange coloration, such as carotenoids. This might be as a result of possible relationships between the processes and mechanisms involved in the synthesis of particular colors and oils.

Conclusion

Calendula officinalis was cultivated in Division of Forest Products and Utilization. Total of 12 accessions were analyzed to determine the best accession of *Calendula officinalis* in terms of growth parameters and oil content. All the 12 accessions were planted at same spacing of 25×25 cm. It was concluded that maximum no. of flowers/plant

(17.11), Weight of fresh flowers/p (41.73 g), Weight of dry flowers/p (9.78 g) was observed in Lal-mandi. Highest oil content (0.472%) was recorded in Fiesta gitana orange. Thus it is recommended to grow Fiesta gitana orange for maximum oil percentage.

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