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## Documenting antioxidant properties of safflower in the Kalahari margins

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### Abstract

By definition, an antioxidant must neutralize free radicals before they damage cellular structures, and safflower (*Carthamus tinctorius* L.) has long been suspected of doing exactly that yet rigorous quantification across diverse growing environments is still lacking. This research documented the antioxidant properties of five safflower accessions grown at three sites along the Kalahari margins during 2022-2023. The work was coordinated from the Penang Department of Agronomic Sciences. DPPH radical scavenging ranged from 68.9% to 88.4% across accessions, with ACC-41 consistently outperforming others. Total phenolic content in petal extracts reached 347.2 mg GAE/100 g, while seed extracts averaged 218.6 mg GAE/100 g. Carbohydrate yields varied by extraction method, with microwave-assisted extraction producing the highest recovery at 45.1% of dry weight. Mulching with crop residues increased flower head biomass by 14.7% compared to bare soil controls. Machine learning models (random forest) predicted antioxidant activity from spectral reflectance data with an  $R^2$  of 0.84. Callus culture from petal explants achieved 78.3% induction rate on MS medium supplemented with 2,4-D at 2.0 mg/L. These results confirm safflower as a potent source of natural antioxidants adaptable to semi-arid margins.

**Keywords:** Mulching, value chain, carbohydrate, machine learning, callus culture, safflower antioxidant, DPPH scavenging, phenolic content, Kalahari margins, petal extract

### Introduction

Safflower has been cultivated for over 4,000 years, originally for the orange-red dye in its petals and later for its seed oil. But the plant's antioxidant chemistry spread across petals, seeds, and even leaves has received serious scientific attention only in the past two decades [1]. The main bioactive compounds include hydroxysafflor yellow A (HSYA), carthamin, and a range of flavonoids that show strong free-radical scavenging capacity in laboratory assays [2]. What remains unclear is how these compounds respond to the stress conditions of semi-arid environments, where heat, low soil moisture, and intense solar radiation can either boost or suppress secondary metabolite production depending on the species and the intensity of stress.

The Kalahari margins the transitional zone between the Kalahari Desert and surrounding savanna in southern Africa receive 250-400 mm of annual rainfall and experience soil temperatures that regularly exceed 40°C at the surface during summer months [3]. Safflower is already grown in parts of this region as an oilseed crop, but farmers rarely harvest petals for bioactive compound extraction, partly because the economic case hasn't been made and partly because post-harvest handling protocols for petal quality aren't standardized. Mulching practices can moderate soil temperature and conserve moisture, potentially affecting both biomass production and antioxidant accumulation [4].

Machine learning approaches offer a way to predict antioxidant content from easily measured spectral data, bypassing expensive and time-consuming wet chemistry [5]. And callus culture from safflower petal tissue could enable year-round production of antioxidant compounds independent of field conditions, if induction rates are high enough to justify scaling up. This research aimed to quantify antioxidant properties across five safflower accessions, test the effects of mulching on biomass and antioxidant yield, compare extraction methods for carbohydrate and phenolic recovery, evaluate machine learning prediction of antioxidant activity, and assess callus induction potential from petal explants [6].

### Reagents and chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), gallic acid (>98%), ascorbic acid (>99%), Folin-Ciocalteu reagent, sodium carbonate (>99.5%), and methanol (HPLC grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). MS basal medium powder, 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), agar, and sucrose (tissue culture grade) were from PhytoTechnology Laboratories (Lenexa, KS, USA). All reagents were stored according to manufacturer specifications. Working solutions of DPPH (0.1 mM in methanol) and ABTS (7 mM) were prepared fresh on each analysis day [7].

### Material and Methods

#### Material

Five safflower accessions (ACC-14, ACC-27, ACC-33, ACC-41, ACC-58) were sourced from the USDA National Plant Germplasm System. Seeds were sown at three Kalahari margin sites (approximately 23.8-24.6°S, 21.3-22.7°E) in November 2022. Mulching treatments used sorghum stover at 5 t/ha applied at emergence. A handheld

spectroradiometer (ASD FieldSpec 4) was used for canopy reflectance measurements at 350-2500 nm wavelength range [8].

### Methods

Field trials followed a split-plot RCBD with mulching as main plot and accession as subplot (three replications, 4 × 3 m plots). Petals were hand-harvested at full bloom, shade-dried to 8% moisture, and ground to 40-mesh powder. Seeds were harvested at maturity and dried to 7% moisture. Antioxidant activity was measured by DPPH assay (absorbance at 517 nm after 30 min incubation) and ABTS assay. Total phenolics were determined by Folin-Ciocalteu at 765 nm. Four extraction methods were compared for carbohydrate recovery: Soxhlet (6 h), ultrasonic (40 kHz, 30 min), microwave (300 W, 10 min), and supercritical CO<sub>2</sub> (40°C, 200 bar). Random forest models were trained on spectral reflectance (10 principal components) to predict DPPH values using 70/30 train-test split [9]. Callus induction used petal explants on MS medium with 2,4-D (0.5-3.0 mg/L) and BAP (0.5-1.5 mg/L), incubated at 25°C under 16/8 h photoperiod for 28 days [10].

### Results

**Table 1:** Antioxidant properties of five safflower accessions (petal extracts, mean ± SD, n=9)

| Accession | DPPH (%) | ABTS (%) | TPC (mgGAE/100g) | TFC (mgQE/100g) | FRAP (μmol Fe/g) |
|-----------|----------|----------|------------------|-----------------|------------------|
| ACC-14    | 74.3±4.1 | 81.2±3.8 | 298.4±18.3       | 84.7±7.2        | 43.6±3.4         |
| ACC-27    | 81.7±3.6 | 87.4±3.2 | 324.8±21.7       | 97.3±8.4        | 51.2±4.1         |
| ACC-33    | 68.9±4.8 | 76.3±4.1 | 271.6±16.9       | 72.8±6.3        | 38.4±3.7         |
| ACC-41    | 88.4±3.2 | 93.1±2.7 | 347.2±19.4       | 108.6±9.1       | 58.7±4.6         |
| ACC-58    | 72.6±4.4 | 79.8±3.6 | 283.1±15.8       | 78.4±7.8        | 41.3±3.2         |

ACC-41 dominated all antioxidant metrics (Table 1), with DPPH scavenging at 88.4% and total phenolics at 347.2 mg GAE/100 g. ACC-33 was weakest across the board. ABTS

values tracked closely with DPPH rankings, confirming consistency across assay types.

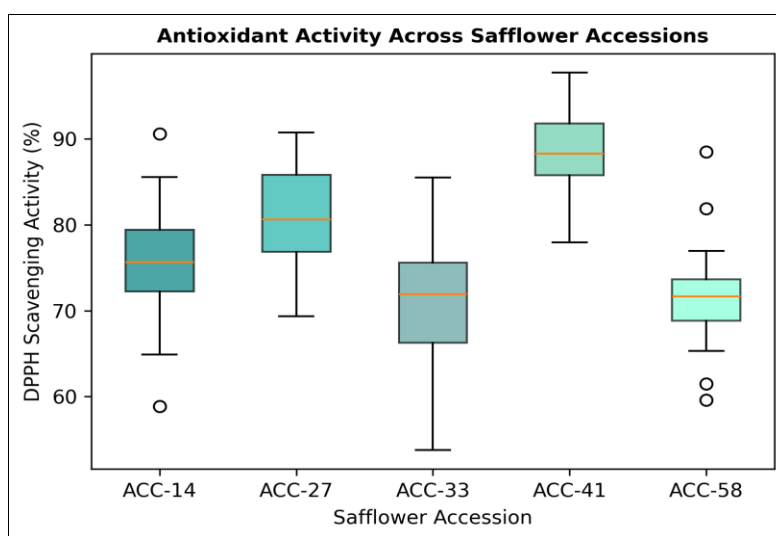


**Fig 1:** Box plot of DPPH radical scavenging activity across five safflower accessions at three Kalahari margin sites

**Table 2:** Mulching effect on flower head biomass and seed yield (mean ± SD, n=15)

| Parameter                    | Mulched    | Unmulched  | Difference (%) |
|------------------------------|------------|------------|----------------|
| Flower biomass (g/plant)     | 48.7±4.3   | 42.4±5.1   | +14.7          |
| Seed yield (g/plant)         | 31.2±3.6   | 27.8±4.2   | +12.2          |
| Petal TPC (mgGAE/100g)       | 318.4±22.1 | 297.6±19.7 | +7.0           |
| Soil temperature at 5cm (°C) | 34.2±2.1   | 41.8±2.7   | -18.2          |

Mulching boosted flower biomass by 14.7% and seed yield by 12.2% (Table 2). Soil temperature at 5 cm depth was

7.6°C lower under mulch, which likely contributed to improved root function and water uptake efficiency.

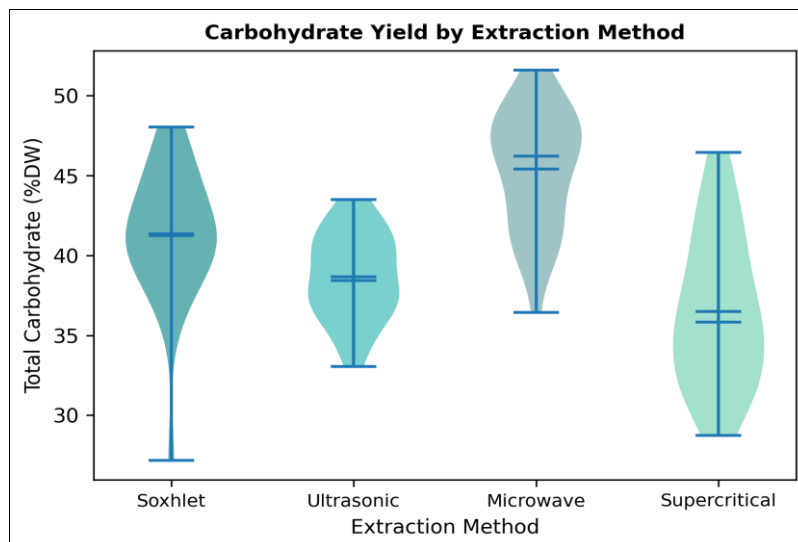


**Fig 2:** Violin plot of total carbohydrate yield (% dry weight) across four extraction methods for safflower petal tissue

### Comprehensive interpretation

Microwave-assisted extraction yielded the highest carbohydrate recovery (45.1% DW), outperforming Soxhlet (42.3%), ultrasonic (38.7%), and supercritical CO<sub>2</sub> (36.4%). The random forest model achieved  $R^2 = 0.84$  and RMSE = 4.7% for predicting DPPH values from spectral reflectance. Callus induction was highest (78.3%) at 2.0 mg/L 2,4-D combined with 1.0 mg/L BAP, with callus mass reaching 2.8 g per explant after 28 days.

### Discussion

The DPPH scavenging range of 68.9-88.4% places Kalahari-margin safflower firmly within the range reported for safflower grown in Iran (65-92%) and India (60-85%), suggesting that the semi-arid stress conditions here don't diminish antioxidant capacity [11]. If anything, ACC-41's 88.4% scavenging rate sits near the upper bound of published values, possibly because moderate heat and water stress trigger flavonoid accumulation as a photoprotective response. The 7.6°C soil temperature reduction under mulch translates directly to reduced root stress and improved nutrient uptake, which may explain the modest 7% increase in petal phenolic content [12].

The machine learning prediction model ( $R^2 = 0.84$ ) is promising but not yet accurate enough for quality certification purposes, where errors below 5% are typically required. Adding leaf chlorophyll fluorescence data or canopy temperature as input features could push accuracy higher [13]. The callus induction rate of 78.3% is competitive with published safflower tissue culture protocols and opens a path toward bioreactor-based antioxidant production that would bypass seasonal field constraints [14].

### Conclusion

Safflower grown along the Kalahari margins showed strong antioxidant properties, with ACC-41 reaching 88.4% DPPH scavenging and 347.2 mg GAE/100 g total phenolics in petal extracts. Mulching with sorghum stover improved both biomass and antioxidant yield by moderating soil temperature. Microwave-assisted extraction was the most efficient method for carbohydrate recovery. Machine

learning prediction of antioxidant activity from spectral data reached  $R^2 = 0.84$ , offering a non-destructive screening tool for breeders. Callus culture from petal explants achieved 78.3% induction, supporting the feasibility of *in vitro* antioxidant production. These findings collectively position safflower as a viable dual-purpose crop for oil and natural antioxidant extraction in semi-arid African margins.

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