

Solvent-Dependent Phytochemical Profile and In Vitro Antioxidant Activity of Chia (*Salvia hispanica* L.) and Flax (*Linum usitatissimum* L.) Seeds

Siddhant Rajendrarao Dhokane

Department of Botany, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India

Abstract

Background: *Salvia hispanica* L. (chia) and *Linum usitatissimum* L. (flax) seeds are recognized as food materials containing diverse phytochemicals and antioxidant-associated constituents. The present study compared solvent-dependent qualitative phytochemical profiles, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical-scavenging activity of seed extracts. **Methods:** Seeds collected in Amravati, Maharashtra, India, during September–October 2023 were shade-dried, briefly oven-dried at 40 °C, powdered, and extracted separately using n-hexane, petroleum ether, chloroform, and glacial acetic acid. Preliminary screening was performed using classical colour and precipitation reactions. TPC was estimated by the Folin–Ciocalteu procedure using catechol as the calibration standard, TFC by an aluminium chloride colourimetric procedure using quercetin as the standard, and DPPH scavenging activity by spectrophotometry. **Results:** Both species showed evidence of multiple phytochemical classes, with solvent-dependent differences. At the 0.2 mg/g sample concentration reported in the project record, TPC in chia ranged from 2.63 to 5.27 mg/g and in flax from 2.90 to 6.18 mg/g across the four solvents. Corresponding TFC values ranged from 17.47 to 23.97 mg/g in chia and 24.97 to 29.97 mg/g in flax. Petroleum ether produced the highest recorded DPPH inhibition in chia (27.65%), whereas n-hexane gave the highest value for flax (17.82%), closely followed by petroleum ether (27.39%). **Conclusion:** The results demonstrate clear solvent dependence in the recovery of colour-reactive phenolics, flavonoids, and DPPH-scavenging activity. Flax showed higher reported TFC across the tested solvents, while chia showed slightly higher petroleum-ether DPPH inhibition. Because independent biological/technical replicate information was not documented in the supplied project record, the quantitative comparisons are presented descriptively

Keywords: *Salvia hispanica*; *Linum usitatissimum*; chia seed; flaxseed; phytochemical screening; total phenolics; total flavonoids; DPPH; antioxidant activity; solvent extraction.

1. Introduction

Plant seeds are important reservoirs of nutritional constituents and biologically active secondary metabolites. Phenolic acids, flavonoids, terpenoids and related compounds can contribute to the antioxidant properties of plant-derived foods, but their apparent recovery depends strongly on the plant matrix, extraction solvent and analytical procedure (Harborne, 1998; Anwar & Przybylski, 2012).

Chia, *Salvia hispanica* L., is an annual herbaceous species of the family Lamiaceae. The accepted taxonomy places it in Kingdom Plantae, phylum Streptophyta, class Equisetopsida, order Lamiales, family Lamiaceae, genus *Salvia* and species *S. hispanica*. Its accepted native range is Mexico to Guatemala, and it is cultivated for food and other uses (Royal Botanic Gardens, Kew, 2026a). Chia seeds contain polyunsaturated fatty acids, dietary fibre and proteins together with polyphenolic constituents. Reported phenolics include caffeic acid, rosmarinic acid, chlorogenic acid, quercetin, myricetin and kaempferol, supporting continuing interest in chia as a functional food and source of antioxidant compounds (Ullah et al., 2016; Grancieri et al., 2019).

Flax, *Linum usitatissimum* L., belongs to the family Linaceae and is classified in Kingdom Plantae, phylum Streptophyta, class Equisetopsida, order Malpighiales, family Linaceae, genus *Linum* and species *L. usitatissimum*. Plants of the World Online recognizes *L. usitatissimum* as an accepted species and records its native range from Türkiye to Iran, with extensive introduction and cultivation elsewhere (Royal Botanic Gardens, Kew, 2026b). Flaxseed contains α -linolenic acid, protein, fibre and a chemically diverse phenolic fraction including lignans and other phenolic compounds. Extraction solvent can markedly influence measured phenolic and flavonoid contents and antioxidant response (Anwar & Przybylski, 2012; Qiu et al., 2020).

The phytochemical composition of chia and flax is relevant to studies of food functionality and plant-derived antioxidants. Qualitative phytochemical screening is a preliminary tool used to indicate broad groups of compounds such as alkaloids, tannins, saponins, terpenoids and proteins; however, such colour and precipitation reactions should be interpreted as screening evidence rather than definitive identification of individual metabolites (Harborne, 1998). For quantitative comparison, the Folin–Ciocalteu method is widely used for operational estimation of total phenolic substances, whereas aluminium chloride-based colourimetry is commonly applied for total flavonoid estimation (Singleton & Rossi, 1965; Chang et al., 2002).

Antioxidant activity can be evaluated using several complementary chemical assays. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a widely used stable-radical method in which antioxidant molecules reduce the radical and produce a measurable decrease in absorbance (Brand-Williams et al., 1995). Published studies have demonstrated DPPH and other antioxidant responses for chia seed extracts, while flaxseed studies have shown that solvent selection affects both phenolic recovery and antioxidant capacity (Ullah et al., 2016; Anwar & Przybylski, 2012).

The present study was undertaken to compare the qualitative phytochemical profile, total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical-scavenging activity of *S. hispanica* and *L. usitatissimum* seed extracts prepared using n-hexane, petroleum ether, chloroform and glacial acetic acid. The specific objectives were to document solvent-wise preliminary phytochemical reactions, quantify Folin-reactive phenolic substances and aluminium-chloride-reactive flavonoids using the calibration procedures recorded in the project, and compare DPPH radical-scavenging activity between the two seed species. The interpretation was restricted to the measurements actually documented in the experimental record.

1.1 Botanical classification of the study species

The accepted classifications used in this manuscript are summarized below. Current taxonomic placement follows Plants of the World Online (Royal Botanic Gardens, Kew, 2026a, 2026b).

Taxonomic rank	<i>Salvia hispanica</i> L.	<i>Linum usitatissimum</i> L.
Kingdom	Plantae	Plantae

Phylum	Streptophyta	Streptophyta
Class	Equisetopsida	Equisetopsida
Order	Lamiales	Malpighiales
Family	Lamiaceae	Linaceae
Genus	Salvia	Linum
Species	Salvia hispanica	Linum usitatissimum



Salvia hispanica



Linum usitatissimum

2. Materials and Methods

2.1 Study material and collection

Seeds of *Salvia hispanica* and *Linum usitatissimum* were collected during September–October 2023 from the area near Malktekdi–Congress Nagar Road, Amravati, Maharashtra, India, as documented in the experimental project record. The material was collected in labelled bags, photographed, cleaned and sorted before processing. Botanical names and family assignments were checked against the accepted taxonomic treatment of the species in Plants of the World Online (Royal Botanic Gardens, Kew, 2026a, 2026b).

2.2 Sample preparation

Cleaned seeds were shade-dried for approximately 10 days and subsequently placed in an oven at 40 °C for 30 min to reduce residual moisture. The dried material was powdered using an electric grinder, passed through a sieve to obtain a relatively uniform particle size, transferred to airtight containers and stored under refrigerated conditions until extraction. Controlled drying and storage are important in preliminary phytochemical work because excessive heat or prolonged exposure to environmental conditions can alter extractable constituents (Harborne, 1998).

2.3 Solvent extraction

For each species, 10 g of powdered seed material was extracted separately with n-hexane, petroleum ether, chloroform and glacial acetic acid. The experimental record describes an 8-day maceration period with regular mixing, followed by filtration through Whatman filter paper. Extracts were protected from light and stored under refrigerated conditions until analysis. Solvent choice and extraction conditions can influence the yield and composition of phenolic/flavonoid fractions, particularly in oilseeds such as flax (Harborne, 1998; Anwar & Przybylski, 2012).

2.4 Preliminary phytochemical screening

Preliminary phytochemical screening was carried out using classical qualitative reactions for alkaloids (Mayer's and Wagner's tests), tannins, saponins (froth test), terpenoids (Salkowski reaction) and proteins (ninhydrin reaction). The symbols used in the original record were retained in the present manuscript: – indicates no detectable reaction, + indicates a positive reaction, and ++/2+ indicates a stronger positive reaction. Classical colour and precipitation reactions are appropriate for preliminary screening, but they do not establish the identity or concentration of individual compounds (Harborne, 1998).

2.5 Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colourimetric approach, following the general principle established by Singleton and Rossi (1965). Catechol was used as the calibration standard in the supplied project record, and absorbance was recorded at 760 nm. The project record gives the calibration equation $y = 0.011x + 1.883$ with $R^2 = 0.9837$. Phenolic concentration was calculated from (absorbance – intercept)/slope. Because catechol rather than gallic acid was documented as the standard, the present manuscript retains the project's reported mg/g units and does not relabel the values as gallic-acid equivalents (Singleton & Rossi, 1965).

2.6 Total flavonoid content

Total flavonoid content was estimated by an aluminium chloride colourimetric procedure using quercetin as the calibration standard, following the established colourimetric principle described by Chang et al. (2002). The project record reports reaction development with sodium nitrite, aluminium chloride and sodium hydroxide and measurement at 510 nm. The recorded calibration relationship was $y = 0.0425x + 0.4069$ with $R^2 = 0.999$. Concentration was calculated from (absorbance – intercept)/slope and expressed in the mg/g units recorded in the project.

2.7 DPPH radical-scavenging assay

DPPH radical-scavenging activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl as the stable radical reagent. The project record states that 50 μ L of extract was mixed with 100 μ L of DPPH solution in a microtitre plate, incubated in the dark and measured at 517 nm. Percentage inhibition was calculated as: DPPH scavenging (%) = [(absorbance of control – absorbance of sample) / absorbance of control] $\times$ 100. The DPPH approach is based on the reduction of the stable DPPH radical by antioxidant compounds (Brand-Williams et al., 1995).

3. Results

3.1 Qualitative phytochemical profile

Both seed species gave positive reactions for several broad phytochemical classes, although the intensity of reactions varied with solvent. In *S. hispanica*, Wagner-positive alkaloid reactions were recorded in all four extracts, while the Mayer reaction was positive in chloroform and glacial acetic acid extracts. Tannins were recorded only in the glacial acetic acid extract. Saponins were detected in n-hexane and petroleum

ether extracts. Terpenoid reactions were positive in all solvents, with the strongest recorded response in n-hexane, petroleum ether and chloroform. Ninhydrin-positive reactions were observed in all four extracts, with the strongest response in glacial acetic acid.

L. usitatissimum showed a broadly similar pattern. Wagner's reagent gave a positive response in all four extracts; Mayer's reagent was positive in chloroform and glacial acetic acid. Tannins were recorded in glacial acetic acid, while saponins were positive in n-hexane and petroleum ether. Terpenoid reactions were positive in all four solvents. Protein-associated ninhydrin reactions were positive in every extract and stronger in glacial acetic acid.

Phytochemical test	n-Hexane	Chloroform	Petroleum ether	Glacial acetic acid
Alkaloids (Mayer)	—	—	+	+
Alkaloids (Wagner)	++	++	++	+
Tannins (Gelatin)	—	—	—	++
Saponins (Foam)	+	2+	—	—
Terpenoids (Salkowski)	2+	2+	2+	+
Proteins (Ninhydrin)	+	+	+	2+

Table 1. Qualitative phytochemical screening of *Salvia hispanica* seed extracts.

Phytochemical test	n-Hexane	Chloroform	Petroleum ether	Glacial acetic acid
Alkaloids (Mayer)	—	—	+	+
Alkaloids (Wagner)	++	++	++	++
Tannins (Gelatin)	—	—	—	++
Saponins (Foam)	+	2+	—	—
Terpenoids (Salkowski)	2+	2+	2+	+
Proteins (Ninhydrin)	+	+	+	2+

Table 2. Qualitative phytochemical screening of *Linum usitatissimum* seed extracts.

3.2 Calibration relationships

Assay	Standard	Wavelength	Recorded equation	R ²
Total phenolics	Catechol	760 nm	$y = 0.011x + 1.883$	0.9837
Total flavonoids	Quercetin	510 nm	$y = 0.0425x + 0.4069$	0.999

Table 3. Calibration relationships.

3.3 Total phenolic content

At the 0.2 mg/g sample concentration used for the cross-solvent comparison in the project record, the highest reported phenolic value for *S. hispanica* was 5.27 mg/g in glacial acetic acid, followed by petroleum ether (4.00 mg/g), chloroform (3.54 mg/g) and n-hexane (2.63 mg/g). For *L. usitatissimum*, the corresponding values were 6.18 mg/g in glacial acetic acid, 3.81 mg/g in petroleum ether, 3.45 mg/g in chloroform and 2.90 mg/g in n-hexane. The pattern indicates stronger recovery of Folin-reactive material in the glacial acetic acid extracts under the reported conditions.

Solvent	<i>S. hispanica</i> (mg/g)	<i>L. usitatissimum</i> (mg/g)
n-Hexane	2.63	2.9
Chloroform	3.54	3.81
Petroleum ether	4.0	3.45
Glacial acetic acid	5.27	6.18

Table 4. Reported phenolic concentration at 0.2 mg/g sample concentration.

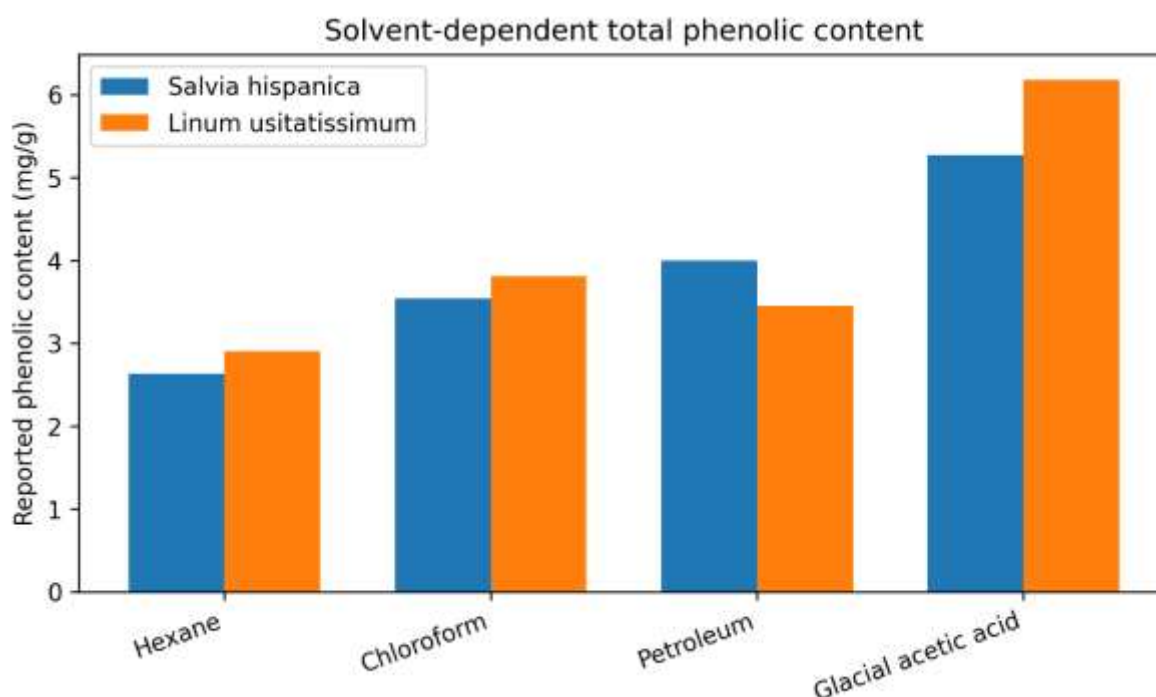


Figure 1. Solvent-dependent total phenolic content using the 0.2 mg/g comparison values.

3.4 Total flavonoid content

At the same 0.2 mg/g comparison point, *S. hispanica* showed flavonoid values of 17.47, 19.14, 20.16 and 23.97 mg/g in n-hexane, chloroform, petroleum ether and glacial acetic acid, respectively. *L. usitatissimum* showed 24.97, 27.47, 27.90 and 29.97 mg/g, respectively. Thus, flax exhibited higher reported flavonoid values than chia in all four solvent comparisons.

Solvent	<i>S. hispanica</i> (mg/g)	<i>L. usitatissimum</i> (mg/g)
n-Hexane	17.47	24.97
Chloroform	19.14	27.47
Petroleum ether	20.16	27.9
Glacial acetic acid	23.97	29.97

Table 5. Reported flavonoid concentration at 0.2 mg/g sample concentration.

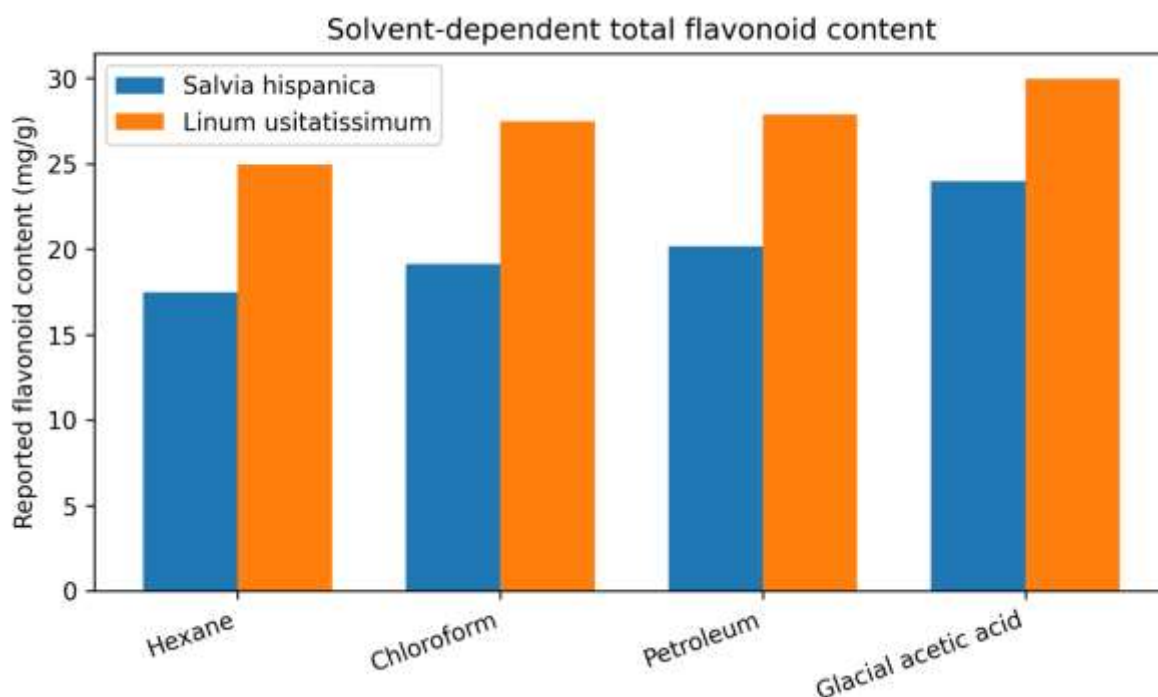


Figure 2. Solvent-dependent total flavonoid content using the 0.2 mg/g comparison values.

3.5 DPPH radical-scavenging activity

The highest DPPH inhibition recorded for *S. hispanica* was 27.65% in petroleum ether, followed by n-hexane (17.69%), chloroform (11.66%) and glacial acetic acid (11.53%). For *L. usitatissimum*, petroleum ether produced 27.39% inhibition, followed by n-hexane (17.82%), glacial acetic acid (11.27%) and chloroform (11.00%). The close petroleum-ether values between the two species indicate broadly comparable DPPH response under the particular assay conditions recorded.

Solvent	<i>S. hispanica</i> inhibition (%)	<i>L. usitatissimum</i> inhibition (%)
n-Hexane	17.69	17.82

Chloroform	11.66	11.0
Petroleum ether	27.65	27.39
Glacial acetic acid	11.53	11.27

Table 6. DPPH radical-scavenging activity recorded for the four solvent extracts.

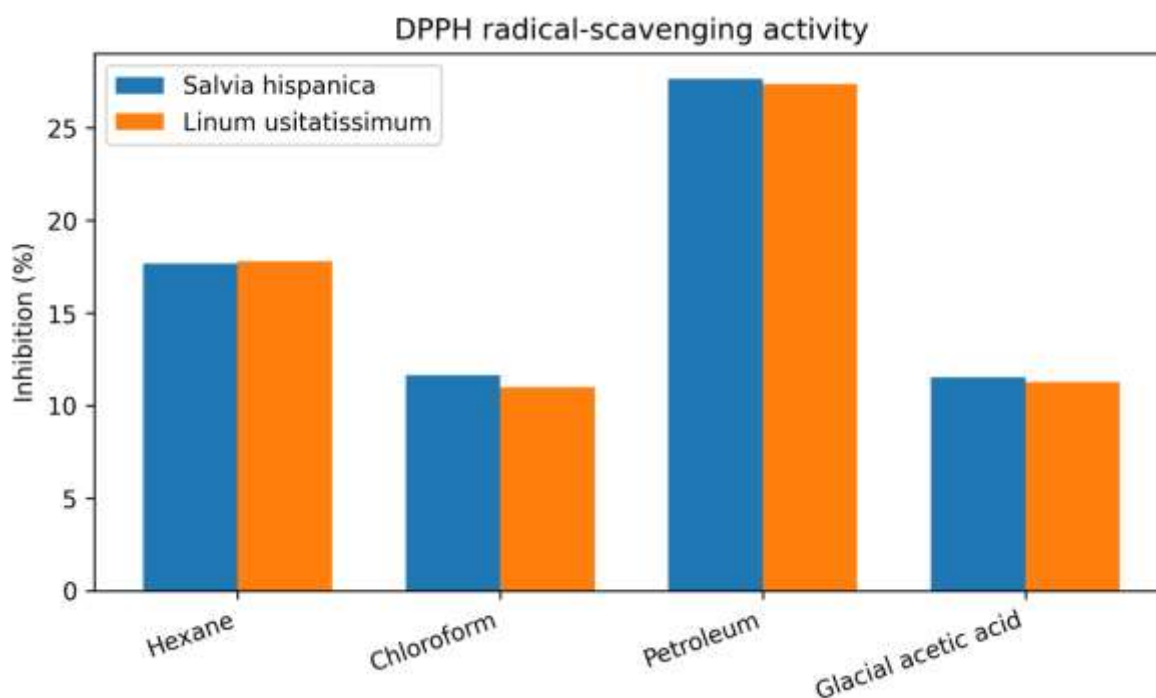


Figure 3. Solvent-dependent DPPH inhibition..

4. Discussion

The present comparison demonstrates that solvent choice substantially influences the apparent phytochemical profile of both seed species. This is expected because secondary metabolites differ in polarity, solubility, matrix association and chemical stability. Previous flaxseed work similarly showed that solvent polarity can change phenolic and flavonoid recovery and antioxidant capacity (Anwar & Przybylski, 2012).

The qualitative screening indicated alkaloid-, tannin-, saponin-, terpenoid- and protein-associated reactions in solvent-dependent combinations. Such tests are useful for preliminary chemical profiling but should be interpreted as class-level screening rather than compound identification. A positive colour reaction can arise from more than one compound and can also be influenced by matrix effects. Modern phytochemical reporting therefore benefits from complementary chromatographic or spectrometric confirmation when individual metabolites are claimed (Harborne, 1998; Fernie et al., 2011).

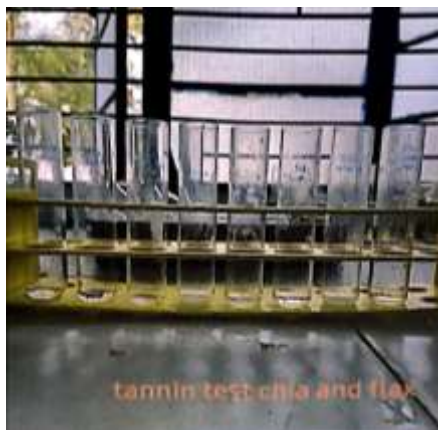
The higher reported flavonoid values of flax relative to chia were consistent across the four solvent comparisons. This observation is directionally compatible with the established chemical diversity of flaxseed, which includes phenolic acids, flavonoids and lignans (Anwar & Przybylski, 2012; Qiu et al., 2020). However, direct numerical comparison with published TPC/TFC values should be avoided unless the calibration standards, extraction ratios, units and dry-weight basis are identical. In the present project, catechol and quercetin were used as the respective standards, whereas many contemporary studies report phenolics as gallic acid equivalents and flavonoids as quercetin or catechin equivalents.

For chia, the strongest recorded phenolic and flavonoid responses occurred in glacial acetic acid, whereas petroleum ether produced the strongest DPPH inhibition. This separation between total colour-reactive content and radical-scavenging activity is not unexpected: antioxidant activity is not determined by a single compound class, and DPPH response can depend on the relative abundance, accessibility and reaction kinetics of multiple reducing molecules. Published studies confirm that chia seeds contain diverse polyphenolic constituents and measurable antioxidant activity, although values vary with extraction and assay design (Ullah et al., 2016; Grancieri et al., 2019; Dib et al., 2021).

The petroleum-ether DPPH responses were similar for the two species (27.65% in chia and 27.39% in flax), while the n-hexane response was slightly higher for flax. These findings should be interpreted as assay-specific inhibition percentages rather than evidence of superior overall antioxidant capacity. DPPH is a useful screening assay, but antioxidant capacity is multidimensional and can be better characterized by complementary methods such as ABTS, FRAP, ORAC or lipid-peroxidation assays (Brand-Williams et al., 1995; Qiu et al., 2020).

An important strength of the present study is that the same solvent set and broadly comparable workflow were applied to both seed species, permitting a direct descriptive comparison. The principal limitation is that the supplied project record does not provide independent biological replicates, technical replicate counts, variability estimates or raw instrument files. Consequently, the manuscript deliberately avoids claims of statistical significance. For publication-quality quantitative phytochemistry, replicate design and transparent reporting of variability are important for reproducibility (Fernie et al., 2011).

Figures :-



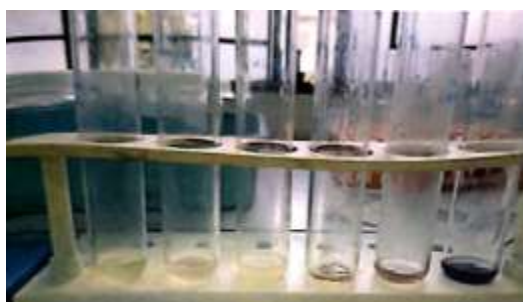
Tannin test of a) Glacial Acetic Acid b) Petroleum c) Hexane d) Chloroform extract.



Saponin test of a) Glacial Acetic Acid b) Petroleum c) Hexane d) Chloroform extract.



Terpenoids test of a) Glacial Acetic Acid b) Petroleum c) Hexane d) Chloroform extract.



Ninhydrin test of a) Glacial Acetic Acid b) Petroleum c) Hexane d) Chloroform extract.



Terpenoids test of a) Glacial Acetic Acid b) Petroleum c) Hexane d) Chloroform extract.

6. Conclusion

Under the extraction and assay conditions documented in the supplied project record, both *Salvia hispanica* and *Linum usitatissimum* seeds exhibited multiple solvent-dependent phytochemical reactions and measurable Folin-reactive phenolic, aluminium-chloride-reactive flavonoid and DPPH-scavenging

responses. Glacial acetic acid produced the highest reported phenolic and flavonoid values in both species at the 0.2 mg/g comparison point, whereas petroleum ether produced the highest DPPH inhibition in both species. Flax showed consistently higher reported flavonoid values than chia across the tested solvents. The results support further investigation of these seeds using replicated assays and compound-specific chromatographic or spectrometric methods.

7. Acknowledgement

The author expresses sincere gratitude to the Department of Botany, Sant Gadge Baba Amravati University, Amravati for providing laboratory facilities and support during the research work. The author is also thankful to Dr. V.N.Nathar Madam, Dr.P.A.Gawande Sir, Dr. K. C. More Sir, Dr. Dhoran Madam for their valuable guidance, encouragement, and support during the completion of this research work.

8. References

1. Anwar, F., & Przybylski, R. (2012). Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Scientiarum Polonorum, Technologia Alimentaria*, 11(3), 293–302.
2. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT—Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
3. Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182. <https://doi.org/10.38212/2224-6614.2748>
4. Dib, H., Seladji, M., Bencheikh, F. Z., Faradji, M., Benammar, C., & Belarbi, M. (2021). Phytochemical screening and antioxidant activity of *Salvia hispanica*. *Journal of Pharmaceutical Research International*, 33(41A), 167–174. <https://doi.org/10.9734/JPRI/2021/v33i41A32314>
5. Fernie, A. R., Aharoni, A., Willmitzer, L., Stitt, M., Tohge, T., Kopka, J., Carroll, A. J., Saito, K., Fraser, P. D., & DeLuca, V. (2011). Recommendations for reporting metabolite data. *The Plant Cell*, 23(7), 2477–2482. <https://doi.org/10.1105/tpc.111.086272>
6. Harborne, A. J. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.
7. Qiu, C., Wang, H., Guo, Y., Long, S., Wang, Y., Abbasi, A. M., Guo, X., & Jarvis, D. I. (2020). Comparison of fatty acid composition, phytochemical profile and antioxidant activity in four flax (*Linum usitatissimum* L.) varieties. *Oil Crop Science*, 5(3), 136–141. <https://doi.org/10.1016/j.ocsci.2020.08.001>
8. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158. <https://doi.org/10.5344/ajev.1965.16.3.144>
9. Ullah, R., Nadeem, M., Khalique, A., Imran, M., Mehmood, S., Javid, A., & Hussain, J. (2016). Nutritional and therapeutic perspectives of Chia (*Salvia hispanica* L.): A review. *Journal of Food Science and Technology*, 53(4), 1750–1758. <https://doi.org/10.1007/s13197-015-1967-0>
10. Grancieri, M., Martino, H. S. D., & Gonzalez de Mejia, E. (2019). Chia seed (*Salvia hispanica* L.) as a source of proteins and bioactive peptides with health benefits: A review. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 480–499.