



A QUALITATIVE EVALUATION: DETECTION OF CARBOHYDRATES (SUGARS) OF LEAF EXTRACT (LE) AND LEAF PROTEIN CONCENTRATES (LPC) OF SELECTED WILD AND CULTIVATED PLANT SPECIES

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ABSTRACT:

Carbohydrates are vital biomolecules in plants, serving key roles in energy storage, metabolic processes, and structural integrity. The present study aimed to qualitatively evaluate sugars in leaf extracts (LE) and leaf protein concentrates (LPC) of selected wild and cultivated plant species using thin layer chromatography (TLC). Carbohydrates were detected in all species, with ten distinct sugars identified based on R_f values and color reactions compared with standards. D (+) – Xylose was the most consistently observed sugar, present in eight species in both LE and LPC. D- (+) – Glucose, D- (-) – Arabinose and D- (-) – Fructose were detected in several leaf extracts, while sucrose was predominant in LPC. Species-specific sugars such as β-D- (+) – Glucose in *Chenopodium album*, lactose in *Brassica oleracea*, and maltose in *Vigna trilobata* were also recorded. In general, leaf extracts (LE) exhibited a wider range of sugars than leaf protein concentrates (LPC), suggesting that some soluble carbohydrates were lost during the protein concentration process. These findings highlight species-specific carbohydrate profiles and confirm the nutritional significance of sugars in both wild and cultivated leafy plants.

Keywords:- Leaf extracts (LE), Leaf protein concentrates (LPC), Carbohydrates, Phytochemical screening Sugars; Thin layer chromatography (TLC); Wild and cultivated plants etc.

INTRODUCTION:

Carbohydrates are the most abundant organic molecules in plants and play an indispensable role in energy storage, structural framework, and metabolic regulation. In leaves, carbohydrates occur as soluble sugars such as glucose, fructose, and sucrose, as well as

storage polysaccharides like starch (Chandel, 2021). They not only serve as essential nutrients but also act as precursors for the biosynthesis of secondary metabolites. Understanding carbohydrate profiles in plant tissues provides valuable insights into their nutritional potential and functional applications.

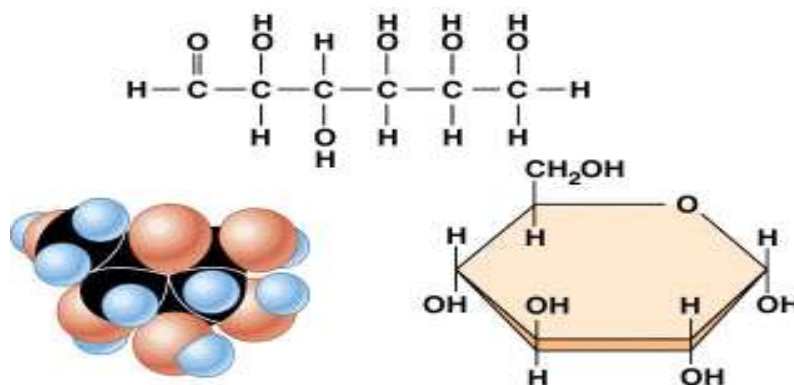


Fig: Structure of Glucose

Source: - (https://courses.worldcampus.psu.edu/welcome/biol011/lesson02_08.html)

Leaf extracts (LE) represent the soluble fraction of leaf phytochemicals, including sugars, while leaf protein concentrates (LPC) are protein-rich products obtained by coagulation and precipitation of soluble proteins (Fernández, et al, 2020). Although LPC is primarily studied for its protein content, it may also retain trace levels of carbohydrates that influence its nutritional and functional quality. A comparative assessment of LE and LPC thus provides insights into the impact of processing on carbohydrate retention and availability (Hernandez, and Hernandez, 1994).

Wild plants, often underutilized, are known to accumulate higher amounts of simple sugars due to ecological adaptations, whereas cultivated leafy vegetables tend to store more starch, reflecting domestication and selection for palatability and yield (Alseekh et al., 2021). Despite their nutritional importance, comparative qualitative studies on sugars in LE and LPC of wild and cultivated species are limited. Such investigations are timely in the context of sustainable nutrition and the utilization of alternative plant resources.

Carbohydrates represent the primary metabolites of plants and are widely distributed in all tissues. Leaves are important sites for carbohydrate synthesis through photosynthesis, where glucose, fructose, and sucrose are produced and translocated to other plant parts. Starch, a storage polysaccharide, accumulates in chloroplasts during the day and is mobilized at night (Smith & Zeeman, 2006). Carbohydrates not only serve as nutritional components but also act as signalling molecules regulating plant growth and stress responses (Ruan, 2014).

Leaf extracts typically contain soluble carbohydrates such as monosaccharides, disaccharides, and small amounts of starch. Several studies have reported that aqueous extracts of spinach (*Spinacia oleracea*), amaranth (*Amaranthus tricolor*), and kale (*Brassica oleracea* var. *acephala*) are rich in soluble sugars (Nemzer et al., 2021; Sarker et al., 2024). Wild leafy plants such as *Chenopodium album* and *Portulaca oleracea* have been shown to contain high levels of glucose and fructose, often exceeding those of

their cultivated counterparts (Grivetti & Ogle, 2000). Such findings suggest that wild plants may serve as valuable reservoirs of dietary sugars.

LPCs are obtained from fresh leaf juice by heat coagulation or acid precipitation of proteins (Pirie, 1971). While primarily studied for their protein content, LPCs also contain varying levels of soluble carbohydrates. However, the concentration process often reduces sugar levels due to separation of the soluble fraction from the protein-rich precipitate (Tenorio et al., 2017). Comparative studies have shown that LPC derived from cultivated species like spinach and amaranth retains lower amounts of sugars compared to crude extracts (Fasuyi, 2005). Information on sugar retention in LPC of wild species remains scarce, highlighting a gap in research.

Wild plants often possess higher concentrations of reducing sugars and monosaccharides due to their adaptation to stress environments and lack of selective breeding (Rosa et al., 2009; Vilkickyte et al., 2019; Jeandet et al, 2022). Cultivated leafy vegetables, on the other hand, are frequently selected for starch accumulation, improved palatability, and higher biomass yield. Studies comparing wild *Amaranthus spinosus* with cultivated *Amaranthus tricolor* have revealed higher sugar diversity in the wild form (Srivastava, 2011). This difference underscores the importance of evaluating both wild and cultivated species for their carbohydrate profiles.

Sugars in leaves contribute directly to dietary energy intake and indirectly to functional food applications. Reducing sugars such as glucose and fructose are rapidly metabolized for energy, while polysaccharides such as starch provide slow-release energy (Kissanga et al., 2021). In addition, soluble carbohydrates act as precursors for glycosides and other bioactive compounds, thereby linking primary and secondary metabolism (Elshafie et al., 2023). The dual role of sugars as energy sources and biochemical precursors underscores their importance in human nutrition.

During the present investigation, ten plant species were selected as protein sources, including cultivated species (*Brassica juncea*

(L.) Czern. & Coss, *Brassica napus* L., *Brassica oleracea* var. *Botrytis* L.) and wild species (*Chenopodium album* L, *Goniocaulon indicum*, (Klein ex Willd). C.B. CL, *Celosia argentea* L., *Vigna trilobata* (L.) Verde, *Digera muricata* (L.) Mart, *Tridax procumbens* L., and *Ocimum americanum* L.) were selected as a protein source. Leaf protein concentrate (LPC) was prepared from the experimental plants following the method of Pirie (1966b). The extract was oven-dried at 50–60 °C and stored for further analysis. For comparison, oven-dried leaf extracts (LE) were also prepared by subjecting freshly expressed leaf juices to the same drying conditions. Both LPC and LE samples were subsequently analysed for qualitative carbohydrate (sugar) composition using thin-layer chromatography (TLC).

Thin Layer Chromatography (TLC) of Sugars:-

a) Sample preparation: Samples were prepared following the method of Singh and Tandan (1970).

b) Preparation of TLC plates: Plates were prepared using a 0.1 M solution of sodium acetate as described by Weidemann and Fischer (1964).

c) Solvent system: The mobile phase consisted of chloroform and methanol in the ratio of 6:4 (Pifferi, 1965).

d) Detection: Sugars were visualized using anisaldehyde-sulphuric acid reagent (Lisboa, 1964).

RESULT & DISCUSSION:

The qualitative screening of carbohydrates i.e. different sugars was carried out in leaf extract (LE) and leaf protein concentrates (LPC) of selected wild and cultivated plant species. The results are presented in tabular and figure form.

Carbohydrate detection in leaf extract (LE) and leaf protein concentrates (LPC) of ten selected wild and cultivated plant species revealed the presence of sugars in all species examined. Thin Layer Chromatography (TLC) analysis, based on R_f values and color reactions, allowed the identification of ten different sugars when compared with authentic standards.

Among these, D (+) – Xylose was the most common, occurring in eight species in both LE

and LPC. Other major sugars detected in leaf extracts included D-(+) – Glucose (Dextrose) in five species, D-(–) – Arabinose in four species, and D-(–) – Fructose (D-levulose) in one species. In contrast, sucrose was more frequently detected in LPC, appearing in five species. Certain sugars showed species-specific occurrence: β-D-(+) – Glucose was identified in *Chenopodium album*, Lactose (milk sugar) in *Brassica oleracea*, and Maltose (4-D-Glucopyranosyl D-Glucose) in *Vigna trilobata*.

In terms of sugar diversity, three sugars were observed in the leaf extract of *Vigna trilobata*, while most other species showed only two sugars in their extracts. The minimum sugar occurrence (only one sugar) was noted in *Brassica juncea*, *Chenopodium album*, and *Celosia argentea*. In LPC, the maximum number of sugars detected was two (in six species), while only one sugar was observed in the remaining species (Table 1a, 1b; Plate 1).

These results clearly indicate that the sugar content of both LE and LPC varied among plant species, with D (+) – Xylose being the most consistently detected sugar across taxa. Furthermore, the reduction in sugar diversity in LPC compared to LE suggests partial loss of soluble sugars during protein concentration.

The findings of the present investigation are in agreement with earlier reports. Barve (1995) reported the presence of sugars such as D-(–) Arabinose, sucrose, D-(–) Fructose, β-D-(+) Glucose, D-(+) Mannose, D-(+) Xylose, and Maltose monohydrate in legume seeds using TLC techniques. Similarly, Bhatia et al. (1972) demonstrated the presence of sucrose, glucose, and fructose in leaf and stem tissues by descending paper partition chromatography. Yemm and Willis (1954), using descending filter paper chromatography, identified glucose and fructose as the predominant sugars in plant extracts and sorghum kernels. Bell (1962) provided additional information on the relative distribution of simple sugars throughout the kingdom of plants.

The present study thus confirms the occurrence of simple sugars in leaves, consistent with previous reports, and additionally highlights species-specific variation in carbohydrate profiles between wild and cultivated plants, particularly in LE versus LPC preparations.

Discussion: - The detection of carbohydrates in all species examined confirms their universal presence in plant leaves. The frequent occurrence of D (+) – Xylose supports its role as a structural sugar, consistent with earlier reports on hemicellulose-derived sugars (Bell, 1962).

Differences in sugar composition between LE and LPC highlight the effect of processing. The reduced sugar diversity in LPC is likely due to the loss of soluble sugars during protein concentration, a finding consistent with Bhatia et al. (1972). However, the retention of sucrose in several LPC samples suggests that some disaccharides co-precipitate with proteins, possibly due to protein-sugar interactions.

Species-specific sugars such as lactose and maltose demonstrate genetic and metabolic influences on carbohydrate profiles, similar to observations by Barve (1995). The detection of glucose and fructose aligns with the findings of Yemm and Willis (1954), who identified them as the predominant plant sugars.

Nutritionally, soluble sugars enhance the energy value of leafy plants. Wild species, which

showed higher variability in reducing sugars, may be better adapted to stress conditions and thus serve as valuable resources for food and nutraceutical purposes.

CONCLUSION: -

The present study demonstrates that carbohydrates are universally present in both LE and LPC of selected wild and cultivated plant species. D (+) – Xylose was the most common sugar, while glucose, arabinose, fructose, sucrose, lactose, and maltose displayed species-specific occurrence. LE consistently exhibited higher sugar diversity compared to LPC, reflecting partial loss of soluble carbohydrates during protein concentration. From a nutritional perspective, the detection of simple sugars in both LE and LPC underscores their importance as potential dietary energy sources. The co-occurrence of sugars with protein in LPC also enhances its nutraceutical value, particularly in regions where wild leafy plants serve as accessible food resources.

These findings provide insights into the biochemical diversity of leafy plants and highlight their nutritional potential.

In the present investigation, density of the L-Arginine solution at isoelectric pH (pH=10.8) were work out from 293.15 K - 313.15 K. It was found that density of solution reduces with raise in temperature and it gain with enhance in molality as depicted in fig.1.

Sound velocity is an essential experimental factor. It provides information regarding extent of molecular interface, stiffness of medium & is greatly exaggerated by the molality & temperature has been studied by Sarvazyan, A. P. (1991). Ultrasound velocity gain with raise in temperature and with concentration in aqueous

medium. The enlarge in ultrasound velocity in any solution indicates the bigger association and stiffness with the molecules of solution . The greater involvement may be due to different types of weak electrostatic forces such as hydrogen bonding, dipole - dipole, ion-dipole, dispersion forces and Van-der Waals forces is cleared by Yadav, M., & Yadav, H. S. (Eds.). (2021). The strong association in the present work is due to intermolecular hydrogen bonding among the amino group of L-Arginine and water molecules as shown in Fig.-2

Table No:-1a. TLC analysis for detection of sugars of leaf extract (LE) of various plants

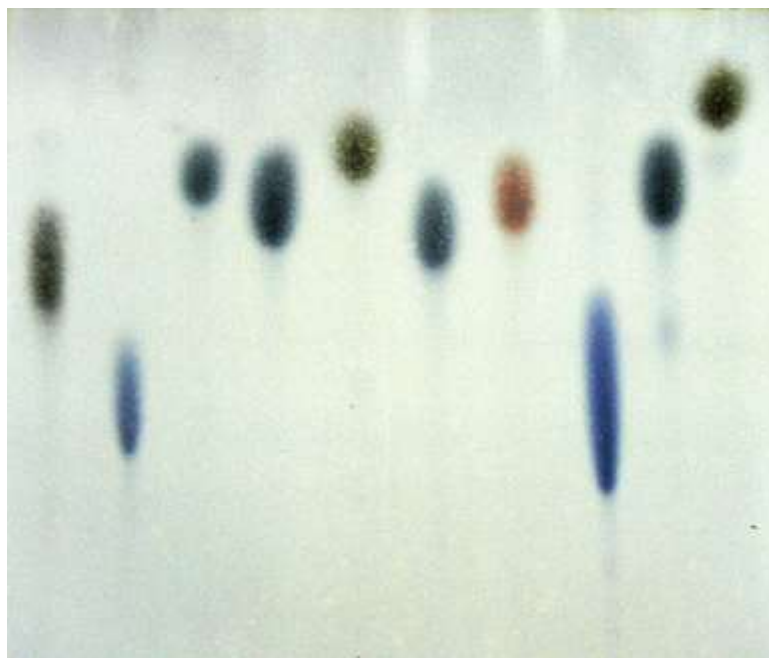
Standard Sugars	Color	Standard (Rf)	<i>Brassica juncea</i>	<i>Brassica napus</i>	<i>Chenopodium album</i>	<i>Gonolobum indicum</i>	<i>Brassica oleracea</i>	<i>Celosi a argentea</i>	<i>Vigna trilobata</i>	<i>Digera muricata</i>	<i>Tridax procumbens</i>	<i>Ocimum americanum</i>
Sucrose	Light green +++	0.62	-	-	-	-	-	-	-	-	-	-
Lactose (Milk Sugar)	(Sky blue) Gray++	0.42	-	-	-	-	-	-	-	-	-	-
B-D-(+)-Glucose	Dark blue gray++	0.75	-	-	-	-	-	-	-	-	-	-
D-(+)-Glucose (Dextrose)	Dark blue gray++	0.72	-	-	-	-	+++	-	+++	+++	+++	+++
D-(-)-Arabinose	Faint gray++	0.8	+++	+++	-	+++	-	-	+++	-	-	-
D-(+)-Galactose	Dark blue gray++	0.68	-	-	-	-	-	-	-	-	-	-
D-(-)Fructose (D-levulose)	Orange+++	0.72	-	++	-	-	-	-	-	-	-	-
Maltose (4-D-Glucopyranosyl D-Glucose)	Dark blue gray++	0.43	-	-	-	-	-	-	-	-	-	-
D-(+)-Mannose (D-Mannopyranose)	Dark blue gray++	0.75	-	-	-	-	-	-	-	-	-	-
D-(+)-Xylose	Light green +++	0.89	-	-	+++	+++	+++	+++	+++	+++	+++	+++
Total Bands			1	2	1	2	2	1	3	2	2	2

High color intensity +++, Intermediate color intensity ++, Low color intensity +

Table No:-1b. TLC analysis for detection of sugars of leaf protein concentrates (LPC) of various plants

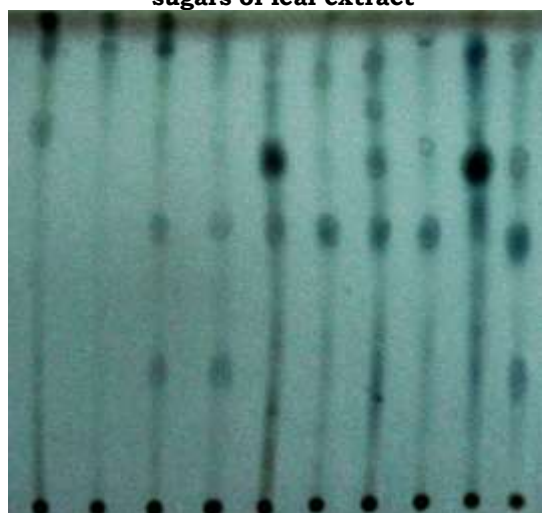
Standard Sugars	Color	Standard (Rf)	<i>Brassica juncea</i>	<i>Brassica napus</i>	<i>Chenopodium album</i>	<i>Gonion indicum</i>	<i>Brassica oleracea</i>	<i>Celosi a argentea</i>	<i>Vigna trilobata</i>	<i>Digera muricata</i>	<i>Tridax procumbens</i>	<i>Ocimum americanum</i>
Sucrose	Light green++	0.62	+++	-	-	+++	+++	-	+++	-	+++	-
Lactose (Milk Sugar)	(Sky blue) Gray++	0.42	-	-	-	-	+++	-	-	-	-	-
B-D-(+)-Glucose	Dark blue gray++	0.75	-	-	+++	-	-	-	-	-	-	-
D-(+)-Glucose (Dextrose)	Dark blue gray++	0.72	-	-	-	-	-	-	-	-	-	-
D-(-)-Arabinose	Faint gray++	0.8	-	-	-	-	-	-	-	-	-	-
D-(+)-Galactose	Dark blue gray++	0.68	-	-	-	-	-	-	-	-	-	-
D-(-)Fructose (D-levulose)	Orange+++	0.72	-	-	-	-	-	-	-	-	-	-
Maltose (4-D-Glucopyranosyl D-Glucose)	Dark blue gray++	0.43	-	-	-	-	-	-	+++	-	-	-
D(+)-Mannose (D-Mannopyranose)	Dark blue gray++	0.75	-	-	-	-	-	-	-	-	-	-
D(+)-Xylose	Light green++	0.89	+++	+++	+++	+++	-	+++	-	+++	+++	+++
Total Bands			2	1	2	2	2	1	2	1	2	1

High color intensity +++, Intermediate color intensity ++, Low color intensity +

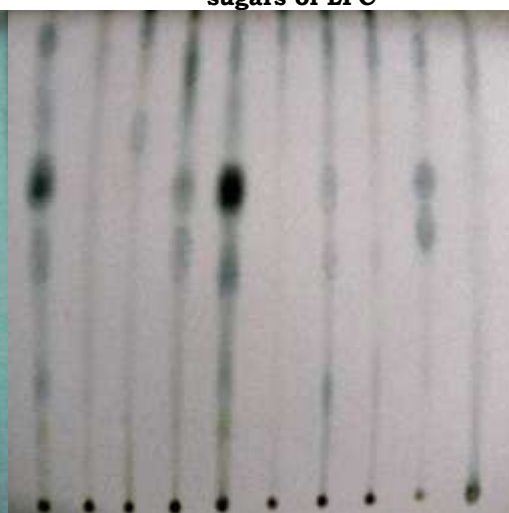
PLATE - 1**TLC analysis for detection of sugar standards**

1 2 3 4 5 6 7 8 9 10

1. Sucrose, 2. Lactose (Milk sugar), 3. B-D-(+) Glucose, 4. D-(+)- Glucose (Dextrose), 5. D-(-) Arabinose, 6. D-(+)- Galactose, 7. D-(-) Fructose (D-levulose), 8. Maltose(4-D-GlucopyranosylD-Glucose), 9. D(+)-Mannose(D-Mannopyranose), 10. D(+)- Xylose.

**TLC analysis for detection of
sugars of leaf extract**

1 2 3 4 5 6 7 8 9 10

**TLC analysis for detection of
sugars of LPC**

1 2 3 4 5 6 7 8 9 10

1. *Brassica juncea*, 2. *Brassica napus*, 3. *Chenopodium album*, 4. *Goniocaulon indicum*, 5. *Brassica oleracea*, 6. *Celosia argentea*, 7. *Vigna trilobata*, 8. *Digera muricata*, 9. *Tridax procumbens*, 10. *Ocimum americanum*.

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