



Effect of Different Solvents and Solvent Mixtures on Extraction of Cashew Nut Shell Liquid (CNSL) and Its Evaluation in Selected Cashew Genotypes

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Abstract

Cashew nut shell liquid (CNSL) is a valuable by-product of the cashew industry. CNSL has diverse industrial applications due to its unique chemical constituents such as anacardic acid, cardanol and cardol. However, inefficient extraction methods limit its commercial utility. Optimization of the extraction solvent is one of the most important factors for the development of efficient CNSL extraction protocols. In this study, we evaluated the effect of different solvents and solvent mixtures on CNSL extraction efficiency. Among the different solvents tested for extraction, acetone, chloroform, isopropanol, methanol, ethanol and two solvent mixtures (ethanol:hexane 1:2 v/v and ethanol:chloroform 2:1 v/v) were found to be the most effective solvents for CNSL extraction. Among these, methanol exhibited the highest CNSL extraction efficiency, which can be attributed to the relatively polar nature of major CNSL constituents such as anacardic acids, cardol and cardanol. Further, CNSL content was evaluated among forty-five cashew genotypes using the best solvent-based protocol and the results showed significant genetic variation in tested genotypes for the CNSL content (10.0-32.6%, $p < 0.05$). The findings of this study pave the way for the development of efficient CNSL extraction protocols. Further, the assessment of CNSL content in the selected accessions will be useful in the cashew breeding programmes aimed at developing cultivars with high CNSL content suitable for industrial purposes and low CNSL types for direct culinary uses.

Keywords: Anacardium, CNSL, Solvent, Industry, Protocol

Introduction

The cashew tree (*Anacardium occidentale* L.) is an important tree species cultivated in more than 30 countries for its edible nuts (Savadi *et al.*, 2020). Cashew nut shell liquid (CNSL) is a significant byproduct of the cashew industry which has wide industrial applications in industries value. Traditionally, CNSL has been widely used in the production of resins, coatings and adhesives and recently is being explored as a source of biodiesel (Biscoff and Enweremadu, 2023), antimicrobial compounds (Souza *et al.*, 2022) and green synthesis materials (Carollo *et al.*, 2024) Traditionally, hot oil bath method is used for commercial extraction of CNSL, in which raw nuts are immersed in a preheated CNSL (180-190 °C) bath for 2-3 minutes, resulting in CNSL

yields of about 7-12% oil (Yusuf *et al.*, 2023). Solvent-based extraction methods are considered to be more efficient for CNSL extraction. However, selection of suitable solvents is an important factor in the development of efficient solvent-based CNSL extraction protocols (Kumar *et al.*, 2017).

However, systematic comparisons of solvents with varying polarity and solvent mixtures for CNSL extraction in cashew remain limited. Furthermore, information on the use of optimized extraction protocols for evaluating CNSL variability in breeding materials is scarce. Therefore, optimizing solvent selection and applying the optimized protocol to diverse cashew genotypes would facilitate accurate phenotyping for breeding as well as industrial utilization.

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Hence, the extraction efficiency of different solvents needs to be evaluated to identify the most suitable solvent. The extraction efficiency of solvents is determined by the solubility of specific lipids or oils in the solvents, which depends on the proportions of polar and non-polar components in the assayed oils (Smedes and Thomassen, 1996; Anthony and Stuart, 2015). Previously, different solvents have been tested in different plant species to identify the most suitable solvent for efficient extraction of seed oils (Tir et al., 2012; Li et al., 2014; Jisieike and Betiku, 2020; Cao et al., 2022). In peony, the study on the effect of different solvents on seed oil extraction showed that a mixture of *n*-hexane and isopropanol (3:2 v/v) solvent of the seven tested solvents yielded the maximum oil from the seeds (Cao et al., 2022). In rubber, seed oil extraction was found to be higher with the *n*-hexane (63.14%) compared to the isopropanol (56.7%) (Jisieike and Betiku, 2020). Traditionally used solvents such as hexane and petroleum ether cause environmental pollution. Recently, alternative solvents that are less toxic and environmentally safe, referred to as “Green solvents” and having extraction efficiency similar to the traditional ones are being explored for oil extractions (Prasad et al., 2022).

Analysis of seed oil contents in the germplasm of different oil-extracting crops is essential for breeding cultivars with higher or modified oils (Uzun et al., 2008; Kumar and Das, 2018; Yinhua et al., 2023). Seed oil analysis in 19 *Jatropha* accessions showed that most of the accessions had seed oil content more than 30% (Kumar and Das, 2018). In sesame, the oil contents in the landraces varied widely from 41.3 to 62.7% (Uzun et al., 2008). The seed oil content in 22 *Acer truncatum* germplasm varied from 24.4% to 39.2% (Yinhua et al., 2023). Despite the great economic significance of CNSL, there are limited studies on the analysis of CNSL contents in cashew germplasm and breeding lines (Muralidhara et al., 2023; Savadi et al., 2025). Wide variations were observed in the CNSL contents of the Indian cashew germplasm analyzed in a previous study (Savadi et al., 2025). The CNSL content varied from 0% to 41.83% in the 24 diverse cashew accessions studied (Savadi et al., 2025). Such variability in the CNSL contents in cashew accessions suggest the potential utility in breeding. However, comprehensive screening of germplasm for this trait remains underexplored in cashew.

The current study aims to understand the effect of different solvents on CNSL extraction and evaluate the CNSL contents in the cashew cultivars and F_1 progenies using the best solvent to identify the variations in the CNSL content. The findings of this study provide a basis for developing efficient CNSL extraction protocols and facilitate the identification of genetically diverse cashew genotypes for breeding programmes targeting both industrial and culinary applications.

Materials and Methods

Plant Material and Experimental Design

Mature raw cashew nut samples of six cultivars, viz. Bhaskara (BK), Brazilian Dwarf (BD), Ulla 3, Vengurla 4 (V4), Vengurla 7 (V7), Vridachalam 3 (VRI3) and 39 F_1 progenies

derived from five different crosses [BD × H130 (UV), BD × Thali (Thaliparamba 1), BD × V7, BK × H-130 (UV), V7 × 116 (NRC116)] were collected from trees planted in the experimental plots of ICAR-Directorate of Cashew Research (ICAR-DCR), Puttur, Karnataka, India (12.45° N, 75.15° E, 90 m above MSL). The study was conducted for two consecutive harvest seasons during 2024-2025.

Harvested nut samples were cleaned by washing thoroughly with detergent and distilled water. Washed nuts were dried in a dryer at 60 °C for 8 h to reduce the moisture content to 8%. Dried nuts were cut open using a manual nutcracker and the cashew nut shells (CNS) were separated from kernels and testa using forceps. The cashew nut shells (CNS) were further cut into small pieces and immediately transferred small cloth bags. The experiment followed the completely randomized design with three biological replicates per genotype.

Solvent Selection

Seven solvents (hexane, acetone, chloroform, petroleum ether, isopropanol, methanol and ethanol) and two binary solvent mixtures (ethanol:hexane 1:2 v/v and ethanol:chloroform 2:1 v/v) representing polar and non-polar classes were evaluated for their extraction efficiency in cashew nut shells of the Bhaskara cultivars. All solvents were of analytical grade (≥99% purity; SRL, India) and stored under room conditions and the solvent mixtures were prepared before the experimental use. The selected solvents represent a broad range of polarities commonly employed in lipid extraction studies, enabling comparison of their extraction efficiencies for the phenolic lipid constituents of CNSL.

CNSL extractions were performed using the Soxhlet method with the Soxhlet apparatus (capacity: 100 mL) (Borosil Scientific, India) following the American Oil Chemists’ Society method (AOAC, 1990) for 8 hours with five grams of sample. The heating mantle of the apparatus maintained gentle boiling and after completion of extraction, solvents were recovered removing the solvent from the extractor. All extractions were carried out under identical extraction time and solvent volume to ensure comparability among treatments. Extracted CNSL was dried in a desiccator overnight and weighed using a weighing balance (Wensar Weighing Scales Limited, India). CNSL extraction was performed in three replications in the solvent screening experiments with the best identified solvent.

CNSL content (%) was calculated as:

$$\text{CNSL \%} = \frac{\text{Weight of extracted CNSL}}{\text{Weight of sample}} \times 100$$

Analysis of CNSL Content in Cultivars and F_1 Progeny

CNSL contents were analyzed in six cashew cultivars (BK, BD, Ulla3, V4, V7, VRI3) and 39 F_1 progenies using the best solvent identified in this study. Three samples (5 g each) of each genotype were analyzed using the protocol described above.

Statistical Analysis

Statistical analyses of the data were conducted using XLSTAT software in the Microsoft Excel spreadsheet. Analysis of

variance (ANOVA) was performed to assess the effect of solvents and CNSL contents in different genotypes. Significant differences among solvent means were determined using Tukey’s Honest Significant Difference (HSD) test at $p \leq 0.05$. The least significant difference (LSD) was also calculated at $\alpha = 0.05$ using the formula:

$$LSD = t_{\alpha/2, df_{error}} \sqrt{MSE \left(\frac{1}{n_A} + \frac{1}{n_B} \right)}$$

for evaluating the significant differences among the genotypes for CNSL contents. The assumptions of normality and homogeneity of variance were examined prior to ANOVA analysis.

Results and Discussion

Identification of suitable solvents is an important factor for maximizing oil extraction from different samples and developing efficient extraction protocols (Dahmer *et al.*, 1989; Danlami *et al.*, 2015; Yusuff, 2021; Keneni *et al.*, 2021). This study establishes solvents suitable for efficient CNSL extraction and analyzes variations in CNSL content among the cashew cultivars and F_1 breeding lines.

Identification of Suitable Solvents for CNSL Extraction

The results of extraction efficiencies of different solvents showed significant differences in extracting CNSL ($p < 0.001$) (Table 1). For CNSL extraction, methanol showed the highest extraction efficiency (30.33%), followed by ethanol: hexane (1:2 v/v) (28.28%), acetone (28.26%) and ethanol (27.60%) (Figure 1). In contrast, hexane and petroleum ether showed significantly lower CNSL yields (17.83% and 18.11%, respectively) (Figure 1). Post hoc tests revealed that except for hexane and petroleum ether, the other seven tested solvents showed significantly higher CNSL extraction efficiency suggesting their suitability for higher CNSL extractions. High extraction with methanol may be attributed to its higher polarity, which enhances the extraction of phenolic lipid constituents, particularly anacardic acids that constitute a major fraction of natural CNSL. These differences in the efficiency of different solvents for CNSL extractions are consistent with the differences in the polarity of CNSL constituents. For instance, CNSL has more polar phenolic compounds such as anacardic acid, cardol and cardanol (Veeramanoharan and Kim, 2024). Results of this study are consistent with other studies on testing solvents efficacy for seed oil extractions (Jisieike and Betiku, 2020; Wang *et al.*, 2022).

Table 1: Analysis of variance (ANOVA) for CNSL extraction yields from the cashew nut shells of Bhaskara (BK) cultivar using different solvents

Source of Variation	Degrees of Freedom	Mean Squares (MS)
		CNSL
Solvents	8	60.12*
Residuals	18	6.96

*Indicates the significant differences at significance level ($p \leq 0.001$)

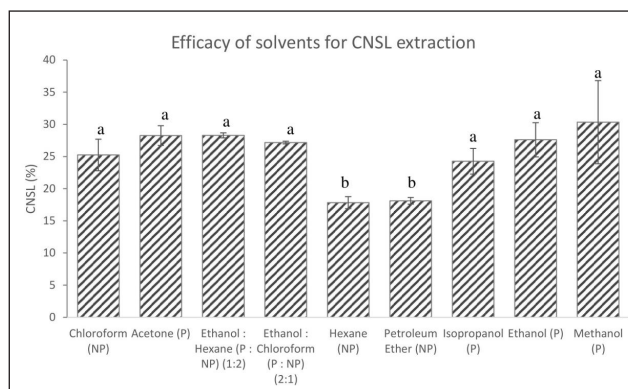


Figure 1: Mean percentage of CNSL extracted using different solvents. The different letters above the error bars represent significant differences ($p \leq 0.05$)

Further, the results of CNSL extraction with the solvent mixtures in this study is consistent with previous studies, which have shown that a mixture of different types of solvents can increase the oil extraction efficiency (Saini *et al.*, 2021; Wang *et al.*, 2022). In rice, the mixture of solvents (isopropanol and cyclohexane 1:1, v/v) gave higher bran oil recovery than the extractions with pure solvents (Wang *et al.*, 2022). In hemp, seed oil extraction with a ternary mixture of solvents (40% n-hexane, 40% 2-propanol and 20% ethyl acetate) gave a higher yield than the extractions with pure solvents (Allay *et al.*, 2024). In peony, a mixture of n-hexane and isopropanol (3:2 v/v) solvent gave high oil yields compared to the other six solvents used in the study (Cao *et al.*, 2022). Although methanol exhibited the highest extraction efficiency under laboratory conditions, ethanol-based solvent systems may offer practical advantages for industrial applications because of their comparatively lower toxicity and improved environmental compatibility. These results suggest that solvent selection must be made by screening different solvents based on the chemical composition of the targeted oil and the mixing of solvents can enhance the extraction efficiency for specific oils.

Analysis of CNSL Contents in Cultivars and F_1 Populations using Optimized Methods

Identification of genetic variations for different phenotypic and biochemical traits in germplasm and breeding material will support the classification and conservation of germplasm and genetic improvement of crop plants (Chen *et al.*, 2023). Further, the use of suitable analytical methods and solvents will facilitate faster and accurate analysis of biochemicals in crop plants (Cao *et al.*, 2022; Bhadange *et al.*, 2024).

In this study, methanol, the best solvent identified, was used for the analyses of CNSL contents in different cashew genotypes. The results showed significant variations in CNSL contents of the tested cashew genotypes ($p < 0.001$) (Table 2). In the CNSL content analysis, the highest mean CNSL content was observed in the BD variety ($32.63 \pm 0.6\%$) and the lowest CNSL content was observed in the progeny 4-10-8 ($V7 \times 116$) ($10 \pm 0.81\%$). Table 2 summarizes the mean CNSL values of the tested cashew genotypes. These findings suggest significant genetic variability in the CNSL contents of the evaluated

Sl. No.	Genotype	Cross/ Cultivar	CNSL (%)	Sl. No.	Genotype	Cross/ Cultivar	CNSL (%)
1.	4-7-4	BD	32.63±0.6	24.	3-11-10	BK × H-130 (UV)	22.3±0.59
2.	3-10-1	BD × H130 (UV)	26.49±1.32	25.	3-11-13	BK × H-130 (UV)	22.35±0.16
3.	3-9-12	BD × Thali	29.76±0.74	26.	3-12-4	BK × H-130 (UV)	22.21±3.12
4.	4-1-2	BD × V7	24.36±2.53	27.	3-12-6	BK × H-130 (UV)	24.12±0.86
5.	4-1-5	BD × V7	23.71±2.63	28.	3-12-11	BK × H-130 (UV)	22.1±1.52
6.	4-1-7	BD × V7	25.71±0.81	29.	4-3-8	Ullal3	25.86±2.63
7.	4-2-6	BD × V7	24.5±4.14	30.	4-6-2	Vengurla4	23.39±0.77
8.	4-2-7	BD × V7	18.93±2.12	31.	4-11-7	Vengurla7	29.57±0.4
9.	4-3-3	BD × V7	16.29±1.21	32.	4-11-4	V7 × 116	18.14±0.4
10.	4-3-5	BD × V7	23.71±0.61	33.	4-11-8	V7 × 116	18.21±3.54
11.	4-3-9	BD × V7	19.14±6.46	34.	4-10-2	V7 × 116	23.21±1.92
12.	4-4-1	BD × V7	27.31±1.61	35.	4-10-3	V7 × 116	26±2.02
13.	4-4-2	BD × V7	27.6±0.52	36.	4-10-4	V7 × 116	22±2.23
14.	4-4-3	BD × V7	24.97±3.84	37.	4-10-8	V7 × 116	10±0.81
15.	4-4-5	BD × V7	26.3±0.95	38.	4-10-10	V7 × 116	20±1.41
16.	4-5-2	BD × V7	28.74±0.13	39.	4-11-3	V7 × 116	20.71±1.82
17.	4-6-8	BD × V7	26.47±1.38	40.	4-12-3	V7 × 116	23.85±6.26
18.	4-6-9	BD × V7	22.52±0.18	41.	4-12-4	V7 × 116	16.5±0.71
19.	4-7-1	BD × V7	24.28±0.62	42.	4-19-1	V7 × 116	19.59±0.74
20.	4-7-3	BD × V7	21.01±0.43	43.	4-20-1	V7 × 116	22.85±1.2
21.	4-7-6	BD × V7	21.33±5	44.	4-20-2	V7 × 116	22.11±0.15
22.	4-7-7	BD × V7	29.92±3.23	45.	4-19-2	Vridachalam3	24.17±0.18
23.	4-19-3	Bhaskara	24.06±0.52			LSD ($\alpha=0.05$)	9.70

Note: CNSL contents are presented as mean \pm standard deviation (SD). Least significant difference (LSD) was calculated at $\alpha = 0.05$

genotypes. Such variations in a trait are a prerequisite for targeted breeding to develop varieties with either high CNSL content for industrial applications or low CNSL types for direct use in culinary applications (Muralidhara *et al.*, 2023; Savadi *et al.*, 2025). The observed variation also indicates the possibility of selecting contrasting parental lines for developing mapping populations and breeding cultivars with different levels of CNSL content according to end-use requirements. The variations observed in CNSL contents among the cashew genotypes in this study are consistent with the results of genetic variability observed for oil content in the germplasm of different crops such as *Jatropha* (Kaushik *et al.*, 2007), safflower (Pahlavani *et al.*, 2012; Kurt *et al.*, 2025), groundnut (Yol *et al.*, 2017).

Conclusion

This study demonstrates that polar solvents, particularly methanol and mixed solvents [ethanol:hexane (1:2 v/v)] showed significantly higher CNSL yields from cashew nut shells compared to the non-polar solvents such as hexane (17.83%). Application of the optimized methanol solvent-based Soxhlet protocol for evaluation of CNSL content in 45 cashew genotypes revealed substantial genetic variation for CNSL (10% to 32.63%). Although methanol proved to be

the most efficient analytical solvent, future studies should evaluate environmentally benign solvents with comparable extraction efficiencies for routine industrial applications. The findings establish an efficient CNSL extraction method and provide baseline information essential for modifying CNSL content in cashew breeding programmes. In the future, research should focus on developing eco-friendly extraction methods using green solvents that provide higher extraction yields, and protocols that are suitable for large-scale screening of breeding material. The identified high- and low-CNSL F_1 progenies could serve as valuable breeding materials for developing cultivars tailored for industrial processing and direct culinary applications, respectively.

Ethical Statement

The authors declare that no generative artificial intelligence tools were used in drafting or preparing this manuscript. The manuscript was written, interpreted and revised solely by the authors, who take full responsibility for its content.

Author's Contribution

SS: Conceptualization, Investigation, Data analysis Writing-original draft, Revision. MK: Investigation. RGB: Investigation, SIG: Investigation, SSC: Investigation, MK: Investigation,

Proof editing, MM: Investigation.

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