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Phytochemical Screening, Spectrophotometric Quantification and GC-MS Profiling of *Jatropha Curcas* Leaf Extract

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ABSTRACT

Jatropha curcas is used in traditional medicine across Africa and Asia, including for liver complaints, but the chemical make-up of its leaves is still not fully described. This preliminary study profiled the chemical groups in a single aqueous leaf extract of *J. curcas* using colour tests, spectrophotometry and gas chromatography–mass spectrometry (GC-MS). Leaves collected in Kaduna State, Nigeria, were dried, powdered and cold-macerated in water, and the extract was screened for ten groups of plant chemicals. Each group was then measured in three technical readings of the same extract, so the values describe this extract only and do not show how much the chemistry varies between plants or batches. Nine of the ten groups were present, and only anthraquinones were absent. Expressed as standard equivalents, carbohydrates were the highest (29.80%), followed by phenols (7.64%), flavonoids (5.19%) and tannins (3.66%), while triterpenes, cardiac glycosides, steroids, alkaloids and saponins were each below 2%. GC-MS assigned seven compounds that the literature associates with antioxidant, anti-inflammatory or liver-related activities, the largest peaks being D-limonene (4.26% of the total peak area) and pentadecanoic acid (1.53%); these identities were not confirmed with reference standards, and the seven peaks together made up only 8.81% of the total peak area. No biological activity was tested, so the results give a chemical starting point for, but no evidence of, liver protection. Independent samples, confirmation of the compounds and biological testing are needed.

Keywords: *Jatropha Curcas,* Phytochemical Screening, Spectrophotometry, GC-MS, Phenols, Flavonoids, Medicinal Plants

INTRODUCTION

Plants have been used as medicine for thousands of years, and many modern drugs began as plant compounds. *Jatropha curcas* belongs to the spurge family (Euphorbiaceae) and grows widely in the warmer parts of Africa, Asia and Latin America. Different parts of the plant, including the leaves, bark, roots and latex, are used in folk medicine, and one review recorded close to a hundred ailments treated with it, among them liver-related conditions (Abdelgadir & Van Staden, 2013).

The usefulness of a medicinal plant depends on the chemicals it contains and on how much of each is present. These amounts are not fixed. They change with the plant part used, the solvent used to extract it, the place where it grew, and the method of analysis (Asuk et al., 2015; Oyetayo & Temenu, 2023). Leaves of *J. curcas* appear to carry a wider range of useful chemicals than the stem bark or roots (Asuk et al., 2015). Reviews of the genus point to flavonoids, phenolic compounds, tannins, saponins and diterpenoids as the main groups behind its reported antimicrobial, anti-inflammatory and liver-protective effects (Sabandar et al., 2013).

Care is needed with this plant because it is also toxic. The seeds are the best-known source of toxicity, and the review by Abdelgadir and Van Staden (2013) covers toxicity alongside the traditional uses. Traditional use records only that a plant is used, not that it works, and few of these claims have been tested in controlled studies. Evidence from roots, seeds or purified compounds should therefore not be assumed to apply to the leaf extract studied here. For example, flavonoids purified from the leaves protected mice against radiation damage (Li et al., 2024). Paracetamol overdose is a well-known cause of liver injury and is widely used as a model for testing natural products (Jaeschke et al., 2011), and natural antioxidants have been reported to reduce this kind of damage (Mahmoud et al., 2020).

Many earlier studies on *J. curcas* use only one or two types of analysis. Asuk et al. (2015) measured chemical groups in the leaf, stem bark and root, and Mahmud et al. (2025) combined colour tests with GC-MS for n-hexane extracts of the stem and root. Within the literature we reviewed, we did not find a report that combined colour tests, spectrophotometric assays and GC-MS on the same aqueous leaf extract, so this work is presented as a preliminary chemical profile. The aim of this study was therefore to screen and measure the main chemical groups in an aqueous leaf extract of *J. curcas* and to use GC-MS to tentatively identify individual compounds in it that the literature associates with antioxidant, anti-inflammatory or liver-related activities. No biological activity was tested.

MATERIALS AND METHODS

Collection and Preparation of the Plant Material

Leaves of *Jatropha curcas* were collected from Kaduna North Local Government Area, Kaduna State, Nigeria. A botanist at the herbarium of the Department of Biological Sciences, Nigerian Defence Academy (NDA), Kaduna, identified and authenticated the plant (voucher number NDA/BIOH202645). The leaves were washed and dried in the shade for at least 14 days until fully dry. They were then ground to a powder with an electric blender and kept in a clean, closed plastic container until use.

Preparation of the Extract

The pulverised leaf sample (500 g), weighed on a Model E scale (Model No. ES0, China), was macerated in 5000 mL of water (cold maceration) and allowed to stand for 24 hours, after which it was filtered. The filtrate was concentrated with a rotary evaporator (BUCHI, Model R110, England) and evaporated to dryness on a water bath (Model B11, China) at 50 °C (Kokate et al., 2009; Evans, 2009; Harborne, 1998; Brain & Turner, 1975). The extract was therefore an aqueous extract. The percentage yield was calculated with Equation 1:

$$\text{Yield (\%)} = (W_e \div W_p) \times 100 \quad (1)$$

Where W_e is the weight of the dried extract and W_p is the weight of the pulverised plant material (500 g). The yield was 4.549 g, which is 0.91% of the plant material. The dried extract was kept in a container before analysis.

Qualitative Phytochemical Screening

The extract was screened for ten groups of chemicals using standard colour tests (Sofowora, 1993; Trease & Evans, 1989). A positive reaction was recorded as present (+) and a negative reaction as absent (−). The test used for each group and the sign of a positive result are listed in Table 1. These are group tests: they show whether a class of chemicals is present and do not identify single compounds.

Quantitative Phytochemical Analysis

Each chemical group was measured with a spectrophotometer. Every measurement was done three times (triplicate). These are technical replicates, that is, three readings of the same extract, and not independent samples. The absorbance of each sample was converted to a concentration using a calibration curve made from a known standard for that group. The method, standard and wavelength used for each group are given in Table 2. Results are expressed as a percentage of the extract by weight on a standard-equivalent basis; for example, phenols are expressed as gallic acid equivalents. A value therefore shows how strongly the extract reacted in the assay compared with the standard, and it is not the weight of a pure compound class. The reagent concentrations, reaction times, blank corrections, calibration ranges and equations, limits of detection and quantification, and recoveries for each assay.

Two points in the naming of the groups need to be made clear. The qualitative test for cardiac glycosides (Keller–Kiliani test) was followed by the Baljet reagent method, which measures cardiac glycosides (Solich et al., 1992), so this group is called cardiac glycosides throughout. The terpenoid group was screened with the Liebermann–Burchard test, but the quantitative assay measures triterpenes only (as ursolic acid equivalents), so it does not cover every terpenoid.

Gas chromatography–Mass Sp

Gas Spectrometry (GC-MS)

The aqueous extract was analysed by gas chromatography–mass spectrometry (GC-MS) to identify its volatile and semi-volatile constituents. The analysis was done at the Multi-User Science Research Laboratory (ABUZ), and the data were acquired and processed with MassHunter software (data file NDA.D, acquired on 19 August 2026; autosampler vial 6, sample multiplier 1). Peaks were integrated automatically with the ChemStation integrator (events file autoint1.e). The mass spectrum at the apex of each peak was searched against the NIST14 mass spectral library, and the three best library matches were recorded for each peak together with their match quality (Qual). The relative amount of each compound was expressed as the percentage of the total peak area (Area %), which shows how large each peak is compared with the others and is not a mass concentration. Because the identities rest on library matches and were not confirmed with reference standards, they are tentative.

Statistical Analysis

For each chemical group the mean, standard deviation (SD) and coefficient of variation (CV) of the three readings were calculated and are reported as descriptive measures of the spread of the readings. The three readings are technical replicates of one extract, and the groups were measured with different assays and standards. No inferential test was therefore used to compare the groups, because such a test would not describe variation between independent samples. The calculations were done in IBM SPSS Statistics version 27.

Ethical Approval

This work forms part of a project that received ethical clearance from the Anti-Corruption and Transparency Unit (ACTU), Nigerian Defence Academy, Kaduna (Reference No. ACTU/NDAPGS/FS/BIS042024/5122; project title: Evaluation of the Antioxidant and Anti-inflammatory Properties of *Jatropha curcas* Extract in a Paracetamol-Induced Hepatotoxicity Animal Model). The plant material was authenticated at the herbarium of the Department of Biological Sciences, Nigerian Defence Academy, and the voucher specimen number is NDA/BIOH202645. The phytochemical work reported here was done on the plant extract only, and no animals were used in this part of the study.

RESULTS AND DISCUSSION

Qualitative Screening

Nine of the ten chemical groups tested were present in the extract (Table 1). Alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpenoids all gave positive reactions. Only anthraquinones were absent.

Table 1: Qualitative Phytochemical Screening of *Jatropha Curcas* Leaf Extract

No.	Chemical group	Test	Sign of a positive result	Result
1	Alkaloids	Dragendorff test	Orange precipitate	Present (+)
2	Cardiac glycosides	Keller–Kiliani test	Brown ring between the layers	Present (+)
3	Saponins	Frothing test	Foam that persists after shaking	Present (+)
4	Phenolic compounds	Lead acetate test	Yellow precipitate	Present (+)
5	Tannins	Ferric chloride test	Blue-black, green or blue-green colour	Present (+)
6	Steroids	Salkowski test	Reddish-brown colour	Present (+)
7	Carbohydrates	Molisch test	Violet ring at the interface	Present (+)
8	Flavonoids	Shinoda test	Pink colour	Present (+)

No.	Chemical group	Test	Sign of a positive result	Result
9	Terpenoids	Liebermann–Burchard test	Green colour	Present (+)
10	Anthraquinones	Bornträger's test	Pink, violet or red colour in the ammonia layer	Absent (−)

Source: Author's research (2026). + = present; − = absent.

Quantitative Screening

The amounts of the ten groups are shown in Table 3 and Figure 1. The three technical readings for each group agreed closely. The largest difference between readings was 0.08 percentage points for phenols (7.60% to 7.68%), 0.02 for tannins and 0.01 for flavonoids, and the other groups did not differ at all to two decimal places. A standard deviation of zero at this precision may simply reflect rounding, so the raw absorbances, calibration results and unrounded readings are also given. The close agreement shows that the readings on this one extract were repeatable; it does not show how much the chemistry varies between plants or batches.

Carbohydrates made up the largest share of the extract (29.80%, as glucose equivalents). Among the groups usually linked to medicinal activity, phenols were highest (7.64%), followed by flavonoids (5.19%) and tannins (3.66%). Triterpenes (1.57%), cardiac glycosides (1.34%), steroids (1.29%), alkaloids (1.13%) and saponins (0.86%) were each present at below 2%. No anthraquinones were detected (0.00%), which agrees with the negative colour test. All values are standard equivalents and should not be read as the weight of pure compounds.

Table 2: Methods used to Measure each Chemical Group

Chemical group	Method (reference)	Standard	Wavelength (nm)
Alkaloids	Bromocresol green method (Shamsa et al., 2008; Sharief et al., 2014)	Atropine	470
Flavonoids	Aluminium chloride method (Zhishen et al., 1999)	Quercetin	510
Saponins	Vanillin–sulphuric acid method (Makkar et al., 2007)	Diosgenin	544
Phenols	Folin–Ciocalteu method (Ainsworth & Gillespie, 2007)	Gallic acid	765
Tannins	Folin–Ciocalteu method (Marinova et al., 2005)	Tannic acid	725
Carbohydrates	Sulphuric acid method (DuBois et al., 1956)	Glucose	715
Steroids	Liebermann–Burchard reaction (Harborne, 1973)	Cholesterol	640
Triterpenes	Vanillin–perchloric acid method (Harborne, 1973)	Ursolic acid	548
Cardiac glycosides	Baljet reagent method (Solich et al., 1992)	Ursolic acid	495
Anthraquinones	Bornträger reaction (Evans, 2009)	Rhein	515

Source: Author's research (2026). Calibration details are given in Supplementary Table S1.

Table 3: Quantitative Phytochemical Composition of *Jatropha Curcas* Leaf Extract (Mean of Three Technical Readings of One Extract; Standard-Equivalent Basis)

Chemical group	Mean (% of extract, standard equivalents)	SD	Coefficient of variation (%)
Carbohydrates	29.80	0.000	0.00
Phenols	7.64	0.040	0.53
Flavonoids	5.19	0.006	0.11
Tannins	3.66	0.012	0.33
Triterpenes	1.57	0.000	0.00
Cardiac glycosides	1.34	0.000	0.00
Steroids	1.29	0.000	0.00
Alkaloids	1.13	0.000	0.00
Saponins	0.86	0.000	0.00
Anthraquinones	0.00	0.000	—

Source: Author's research (2026).

Values are percentages of the extract by weight, expressed as equivalents of the standard in Table 2 (carbohydrates as glucose, phenols as gallic acid, flavonoids as quercetin, tannins as tannic acid, triterpenes as ursolic acid, steroids as cholesterol, saponins as diosgenin, alkaloids as atropine, anthraquinones as rhein, and cardiac glycosides as ursolic acid). The readings are three technical replicates of one extract. SD = standard deviation; CV = SD ÷ mean × 100. An SD of 0.000 means the readings were identical at the reported precision (raw data in Supplementary Table S2).

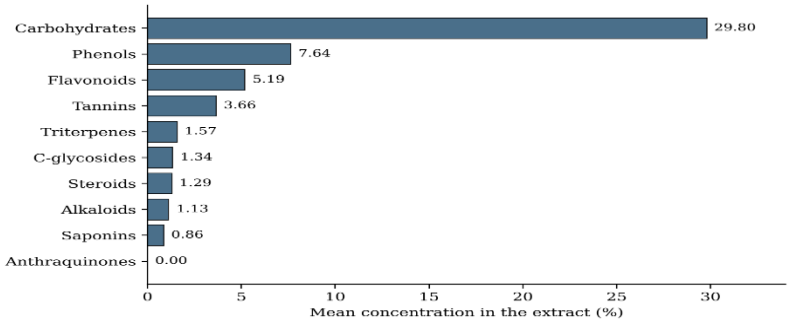


Figure 1: Amounts of the ten chemical groups in the *Jatropha curcas* leaf extract (mean of three technical readings; standard-equivalent basis)

Because the three readings are technical replicates of one extract and the groups were measured with different assays and standards, no statistical comparison between groups was made. The differences in amount between groups are described, not tested.

GC-MS Profile

GC-MS library matching tentatively assigned seven compounds that have been associated in the literature with antioxidant, anti-inflammatory or liver-related activities (Table 4 and Figure 2). D-Limonene had the largest peak among them (4.26% of the total peak area), followed by pentadecanoic acid (1.53%) and eucalyptol (0.85%). The other four compounds, 1H-indole, patchouli alcohol, alloaromadendrene and O-cymene, each accounted for between 0.49% and 0.58%. Together, the seven peaks made up only 8.81% of the total peak area, so they are not the major constituents of the extract. They were selected for Table 4 because of their reported activities and not because of their size. The complete peak list, with retention times, match quality and peak areas, is given in Supplementary Table S3, and the chromatogram is given in Supplementary Figure S1. Other peaks were also seen. Cyclosiloxanes, trichloroacetic acid and esters such as isopropyl palmitate may come from the solvent, the column or the handling of the sample, and they are listed in Supplementary Table S3. Naphthalene (0.38%) and anthracene (0.09%) were also detected, and their significance is discussed in section 4.2.

Table 4: Compounds Tentatively Identified by GC-MS in the *Jatropha Curcas* Leaf Extract that Have Been Associated with Antioxidant, Anti-Inflammatory or Liver-Related Activities in the Literature

No.	Compound	RT (min)	Formula	Peak area (%)	Evidence grade	Reported activity in the literature (model and source)
1	D-Limonene	12.422	$C_{10}H_{16}$	4.26	Direct (animal model)	In a carbon tetrachloride rat model, D-limonene prevented the rise in liver enzymes, kept glutathione and antioxidant enzymes near normal, and lowered markers of lipid damage (malondialdehyde), fibrosis (hydroxyproline) and inflammation (NF-κB) (Ahmad et al., 2018)
2	Pentadecanoic acid	28.818	$C_{15}H_{30}O_2$	1.53	Limited direct	Serum pentadecanoic acid was lower in patients with more active fatty liver disease, and mice on a methionine- and choline-deficient diet given it had lower AST and fewer ceroid-laden liver macrophages (Yoo et al., 2017)
3	Eucalyptol (1,8-cineole)	12.996	$C_{10}H_{18}O$	0.85	Direct (animal model)	In lead-exposed rats, 1,8-cineole lessened liver injury by lowering oxidative stress and inflammation and modulating TLR4/MyD88/NF-κB signalling (Abdollahi et al., 2024)
4	1H-Indole	31.200	C_8H_7N	0.58	Indirect	In cell studies, gut-derived tryptophan metabolites, including indole, modulated inflammatory responses in liver cells (hepatocytes) and macrophages (Krishnan et al., 2018); evidence is cell-based and indirect
5	Patchouli alcohol	26.439	$C_{15}H_{26}O$	0.57	Indirect	Tested as whole patchouli oil, of which patchouli alcohol is a major constituent: the oil lowered liver enzymes, tissue damage, oxidative stress and fat accumulation in ethanol-injured rat liver (Huang et al., 2018); not tested as the pure compound
6	Alloaromadendrene	22.463	$C_{15}H_{24}$	0.53	Indirect	No liver-specific study was found; antioxidant and anti-inflammatory activity has been reported only for essential oils rich in sesquiterpenes (Abd-ElGawad et al., 2021)
7	O-Cymene	12.902	$C_{10}H_{14}$	0.49	Indirect	Liver data are for the isomer p-cymene, which protected liver cells from ethanol-induced death and reduced fibrosis in cell and rat models, with higher SOD and GSH (Atif et al., 2025); activity of o-cymene itself is not established
	Total			8.81		

Source: Author's research; GC-MS analysis (2026), RT = retention time.

The compounds are tentative library matches and were not confirmed with reference standards. Peak area is relative abundance in the chromatogram, not absolute concentration. Evidence grades: Direct (animal model) = tested for liver outcomes in an animal model of liver injury; Limited direct = liver outcomes reported from patient association data and one animal study; Indirect = only cell-based, antioxidant or anti-inflammatory data, whole-oil data, or data for a related compound. The activities are literature reports on these compounds, mostly on pure samples in other models; they are not shown for this extract and do not demonstrate liver protection.

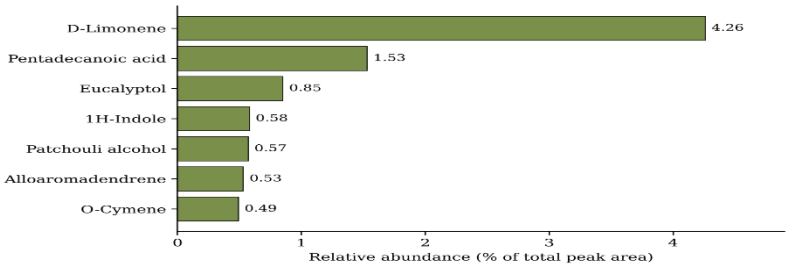


Figure 2: Relative abundance (% of total peak area) of the seven tentatively identified compounds that have been associated with antioxidant, anti-inflammatory or liver-related activities. Peak areas are not concentrations

Discussion

What the Extract Contains

The leaf extract contained nine of the ten chemical groups tested. This is in line with earlier work on the genus, which lists diterpenoids, flavonoids, phenolic compounds, tannins and saponins as the main groups (Sabandar et al., 2013), and with the report that *J. curcas* contains diterpenoids, flavonoids, phenols and other classes (Abdelgadir & Van Staden, 2013). Asuk et al. (2015) also found a wide range of chemical groups in the leaves, which fits our finding of a varied profile.

Carbohydrates formed the largest share of the extract at 29.80%. These are mostly plant sugars and are better seen as part of the plant's basic nutrition than as the main active part. Phenols (7.64%), flavonoids (5.19%) and tannins (3.66%) are of more interest. Phenols and flavonoids are the chemicals most often linked to antioxidant activity, meaning the ability to neutralise harmful reactive molecules that damage cells. Papalia et al. (2017) reported a flavonoid-rich leaf extract of *J. curcas* with strong antioxidant and cell-protective activity, and Li et al. (2024) isolated six main flavonoids from the leaves, including vitexin and isovitexin, that protected mice from radiation damage. Our results show that the extract contains the same classes of chemicals, which makes it reasonable to test its antioxidant effects directly.

It is difficult to compare our percentages with those of other studies. Asuk et al. (2015), for example, reported up to 7.35% flavonoids in an ethyl acetate leaf extract and 4.62% polyphenols in a methanol extract, whereas the present extract was made with water, and the assays, standards and reporting bases also differ. Mahmud et al. (2025) used successive n-hexane, chloroform, ethyl acetate and methanol extracts of the stem and root and ran GC-MS on the n-hexane extracts only. Extracts of different polarity carry different compounds, so such results cannot be compared directly with ours. Amounts depend strongly on the solvent, the plant part, the growing conditions and the method used (Asuk et al., 2015; Oyetayo & Temenu, 2023), so differences between studies are expected and do not by themselves point to an error in any of them.

The three readings for each group were almost identical, which shows that the assays were repeatable on this extract. The readings were repeated measurements of one extract, however, so they say nothing about how much the chemistry might vary between plants, places or seasons. The profile is uneven, with a few groups present in larger amounts and several present only in small amounts, but this is a description and not a tested difference. The percentages are standard equivalents from group assays. A figure such as 7.64% for phenols or 5.19% for flavonoids is therefore not the weight fraction of phenolic compounds or flavonoids in the extract, because the assays also react with other substances and no validated conversion from standard equivalents to weight is available.

What GC-MS Adds

The colour tests and spectrophotometry measure whole groups of chemicals. GC-MS looks at individual volatile compounds, so it adds a second and more detailed view. Of the seven tentatively identified compounds that have literature links to antioxidant, anti-inflammatory or liver-related activities, D-limonene had the largest peak, but the seven peaks together made up only 8.81% of the total peak area and should not be called the main constituents of the extract. The published support differs from compound to compound (Table 4). D-Limonene and eucalyptol have been tested for liver outcomes in animal models of chemically induced liver injury (Ahmad et al., 2018; Abdollahi et al., 2024), and pentadecanoic acid has limited direct data from patients and one mouse model (Yoo et al., 2017). The support for 1H-indole, patchouli alcohol, alloaromadendrene and O-cymene is indirect, because it comes from cell studies, a whole oil, essential oils or a related compound (Krishnan et al., 2018; Huang et al., 2018; Abd-ElGawad et al., 2021; Atif et al., 2025). Antioxidant and anti-inflammatory findings are indirect evidence and should be kept apart from direct protection of the liver.

Two points should be kept in mind. First, GC-MS detects only volatile and semi-volatile compounds. The phenols and flavonoids that dominate Table 3 are not captured by this method, so the two profiles complement each other and cannot be swapped. Very volatile compounds such as D-limonene and eucalyptol may have been partly lost when the extract was concentrated at 50 °C, so their peak areas are not their amounts in the fresh leaf. Second, the activities listed in Table 4 come from published studies on these compounds, often on pure samples in other models. GC-MS shows that a compound is probably present and roughly how large its peak is, but it does not show that the compound is active in this extract.

Naphthalene and anthracene were also detected (0.38% and 0.09% of the total peak area). They are polycyclic aromatic

hydrocarbons that are of toxicological concern at high exposure, but a peak area is a relative abundance and not a dose, so it cannot be used to judge the safety of the extract. These compounds can come from environmental deposition on leaves, from burning, or from contamination during handling or analysis, so their source in this sample is unknown. Their presence is a reason to repeat the analysis with blanks and reference standards, and to measure them quantitatively, before the extract is considered for any use in people. The toxicity reported for *J. curcas* more generally (Abdelgadir & Van Staden, 2013) is a further reason for caution.

Limitations

This study has several limits. Only one batch of leaves from one location was analysed, and the three readings are technical replicates of one extract, so the results describe this extract and not the plant in general. The percentages are standard equivalents from group assays and are not the weights of pure compounds. Only water was used as the extraction solvent, so compounds that dissolve poorly in water may be under-represented, and the results cannot be compared directly with ethanol or methanol extracts. The colour tests show only whether a group is present, and the terpenoid screening and the triterpene assay do not cover the same set of compounds. GC-MS identities came from library matching and were not confirmed with pure reference standards or retention indices, and some peaks may be artefacts of the solvent or the equipment. The activity of the compounds was taken from the literature and was not tested here, and the safety of the extract was not assessed. Finally, the extract was not separated into fractions, so we cannot say which compounds are responsible for any biological effect. The results should therefore be read as an exploratory chemical profile.

Next Steps

Future work should analyse independent batches or plants so that variation can be estimated, confirm the main compounds against reference standards, use HPLC or LC-MS to profile the phenols and flavonoids that GC-MS cannot detect, compare leaves from different places and seasons, and test the extract in biological models, ideally with the extract split into fractions to find the active parts.

CONCLUSION

The aqueous leaf extract of *Jatropha curcas* examined in this single-batch, technical-replicate study contained nine of the ten chemical groups screened, with carbohydrates, phenols, flavonoids and tannins the highest as standard equivalents. GC-MS library matching tentatively assigned seven compounds, led by D-limonene and pentadecanoic acid, that have antioxidant, anti-inflammatory or liver-related activities reported in the literature. These results give a preliminary chemical basis for further work, but they do not show that the extract protects the liver. Independent samples, confirmation of the compounds with reference standards and biological testing are needed.

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