

Evaluation of Scaling Inhibiting Compounds in *Cajanus cajan* **and** *Vigna subterranea* **Shell Extracts for Industrial Utilization**

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ABSTRACT: The objective of this paper is to evaluate and present the scaling inhibiting compounds of aqueous and methanolic extracts obtained from cajanus cajan (*c. cajan*) and *vigna subterranean* (*v.subterranea*) shells collected from Nsukka, Enugu State, Nigeria using appropriate standard techniques. Quantitative phytochemical analysis (expressed in mg/100g) revealed the following secondary metabolites for *C. cajan:* flavonoids (2226.50± 47.35), phenolics (6294.65±117.35), saponins (2.53±0.15), alkaloids (587.42±42.3), steroids (0.77±0.02), terpenoids (989.87±26.72), and tannins (176.49±13.18). Similarly, *V. subterranean* exhibited; flavonoids (2226.50± 47.35), phenolics (6400.11±65.22), saponins (1.79±0.4), alkaloids (114.22±17.64), steroids (0.46± 0.06), terpenoids (308.94±10.22), and tannins (58.18±1.12). GC-MS analysis of both *C. cajan* and *V. subterranean* extracts revealed 14 peaks of different compounds which includes; phenol, methylphenol, dimethylphenol, 2-furaldehyde, 2 hydroxymethifuran, levoglucosan, 4-mehtylguaiacol, vinylphenol, 4-vinylguaiacol, eugenol, vanillin, isoeugenol, 4 allyl-2-6dimethoxphenol and dimethylbenzene. Additionally, FT-IR spectra identified functional groups such as O-H (phenolics) at 3438 and 3430, CH² stretching aliphatic at 2923 and 2884, and C=C unsaturated at 1635 and 1643, present in both extracts. The results from GC-MS, FT-IR, and phytochemical studies collectively suggest that these extracts contain environmentally friendly constituents, particularly higher concentrations of phenolic and foaming agents. This supports the potential of *C. cajan* and *V. subterranean* as candidates to be deployed as environmentally friendly scale inhibitors.

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The petroleum industry faces a significant challenge with pipeline blockages caused by the continual accumulation of scale on pipe walls. This growth results from the presence of dissolved calcium and magnesium salts in the flowing fluids. These blockages lead to various issues within the pipeline including pipeline corrosion attacks, reduced fluid flow rates, energy and resource wastage, and ultimately complete line blockage which necessitates facility shutdown (Rostron, 2018a; Sanni, 2016). Historically, addressing scale growth has involved mechanical methods or the use of synthetic chemicals to control or inhibit scaling, both of which are costly and can have adverse effects on aquatic environments if the discharge is not properly managed (Abderrahmane *et al*., 2001; Charlotte Martinod, 2008; Rostron, 2018b). Conventional inhibitors like phosphonates, polyphosphates, and polyelectrolytes have been employed to inhibit scaling. While effective at low concentrations under proper application, these inhibitors have faced criticism due to the release of chemicals like nitrogen, phosphorus, and nonbiodegradable polymers, posing environmental risks. Recently, environmentally friendly synthesized inhibitors such as polyacrylic acids, polymaleic acids, polyaspartic acid, and carboxymethyl insulin have emerged. However, due to the availability and cost of these synthesized chemicals, there's a growing interest in exploring plant-based inhibitors as viable alternatives. Numerous studies have explored the use of agro-based materials and plant extracts, including tobacco, fig leaf, palm leaf, olive leaf, soybean, and paronychia agenttea, to control scale growth in petroleum pipelines and industrial facilities (Abd-El-Khalek *et al.,* 2016; Abd-El-nabey *et al.,* 2020; Abdel-Gaber *et al.,* 2008; Abdel-Gaber *et al.,* 2011; Aidoud *et al.,* 2017; Belarbi *et al.,* 2014; Chaussemier *et al.,* 2015; Kadiri *et al.,* 2018; Khaled, Rahal, *et al.,* 2020). Our focus is on identifying plant materials that have no adverse effects on aquatic environments or the food chain and are consistently available for industrial use. The global availability of *C. cajan* and *V. subterranean* motivates our search for sustainable applications in the industry. *C. cajan* and *V. subterranean* are commonly cultivated for their high protein value in seeds. Although the seeds and leaves of these plants have demonstrated medicinal applications (Dewick, 2009; Gautam and Jachak, 2009; Odu *et al*., 2023; Wang *et al*., 2008), our interest lies in their underutilized shells. Previous studies have confirmed the safety of these plant parts for both humans and animals (A'yuni *et al.,* 2022; Mallikarjuna *et al.,* 2011; Sameer Kumar *et al.,* 2017). Investigating the potential of *C. cajan* and V. *subterranean* shell extracts to produce free radicals for scaling control in the petroleum industry is crucial. High antioxidant value materials, such as those found in plant extracts, are often incorporated into industrial products like polymers, fuels, and lubricants to enhance their performance and longevity, reducing adverse effects on aquatics and petroleum products (Hajizadeh *et al.,* 2019). Agro materials rich in phenolic compounds and foaming agents have shown promise in scaling and corrosion inhibition (Hajizadeh *et al.,* 2019; Kadiri *et al.,* 2018; Khaled, Rahal, *et al.,* 2020; Tan *et al.,* 2023). Harnessing these shells in scale and corrosion industries can increase their economic value and contribute to environmental cleanliness. The shift towards plant-based alternatives is driven by the costs involved in mechanical scale removal and waste

management from synthetic inhibitors, highlighting the potential of agricultural-based materials like *Cajanus cajan* and *Vigna subterranean* shells in scaling inhibition, improving economic value, and reducing environmental impact. Hence, the objective of this paper is to evaluate and present the chemical composition of aqueous and methanolic extracts obtained from *Cajanus cajan* and *vigna subterranean* shells collected at Nsukka, Enugu state Nigeria.

MATERIALS AND METHODS

The shells of *cajanus cajan* and *vigna subterranea* were collected at a farmers dumpsite located at Nsukka, Enugu State, Nigeria

Extraction Procedure: Both shells of *cajanus cajan* and *vigna subterranean* were air-dried for four days and then ground into a powder. Approximately 250 grams of each ground samples was weighed and soaked in a flask containing 500 mL of methanol solution. The mixture was shaken, covered with foil and allowed to stand for 24 hours. After this period of 24 hours, the mixture was shaken again and filtered using Whatman filter paper No.1. The filtrate containing the extracts was then processed using a rotary evaporator to recover the methanol solvent. The residual methanol was allowed to evaporate by allowing the container open, leaving behind the dried extract, which was then scraped and stored in a sample container for further analysis.

GC.MS Sample Analysis: A Perkin Elmer Turbo Mass Spectrophotometer of Norwalk model CTO6859 USA was used to determine the molecular weight, elemental compositions, and molecular structure of the *cajanus cajan* and *vigna subterranean* shell extracts. This instrument was equipped with a Perkin Elmer Auto sampler XLGC. Next, a 30 m by 0.25 mm Perkin Elmer Elite-5 capillary column containing 95% dimethyl polysiloxane and a 0.25 mm film thickness was used in equal measure. A 1 μl sample injection volume was used, and the carrier was helium gas flowing at a rate of 0.5 mL/min.

The oven's temperature of operation is set to rise to 280°C at a rate of 20°C, with a 5-minute hold after the temperature was set at 110°C for 4 minutes and the inlet temperature set at 250°C. The analysis took 90 minutes to complete, including a 5-minute wait. The temperature was initially maintained at 180°C, and the MS transfer line was held at 200°C. The GC-MS analysis used electron impact ionization at 70 eV, and total ion count (TIC) was used to assess the data in order to identify and quantify the compounds. The component spectra were compared to the GC-MS library's database of known spectra, and data was processed using Turbo-Mass OCPTVS-Demo SPL software.

FT-IR Analysis of the Extract: A quantity of 0.4 g of KBr was weighed and ground into a powder. A small amount (0.001 g) of the shell sample was then weighed and mixed thoroughly with the ground KBr. The mixture was then molded into a disc shape. This molded disc was placed into the sample compartment of the infrared instrument. Using the Perkin Elmer 3000 MX spectrometer, the scan button was pressed to initiate the scanning process. The IR spectrum and

data were generated over a range of 4000 to 400 cm⁻¹. A total of 32 scans with a resolution of 4 cm^{-1} made up each spectra. Spectroscopic software, Win-IR Pro Version 3.0, was used to examine the IR spectra. The peak sensitivity was set to 2 cm⁻¹.

Preliminary Phytochemical Analysis of the Crude Methanol Extract: Samples were subjected to both qualitative and quantitative phytochemical analysis using the techniques described by Harborme (1998) and Sofowora (1999).

Test	Methods	Observations
Detection of	1 mL of dilute HCL and Dragendroff's was	The appearance of orange-
alkaloids	added in 3 mL of the shell filtrate and the	brown precipitates Indicates
Dragendroff test	mixture was shaken thoroughly.	the presence of alkaloids.
Wagners reagent	2 mL of shell filtrate was added to 1 mL of	Reddish-brown precipitate
test	dilute HCL and Wagner's reagent. The	indicates formation the
	mixture was shaken very well.	presence of alkaloids.
Detection of	1mL of shell extract was added to a small	The yellow precipitate was
Flavonoids	quantity of lead acetate solution.	formed which indicates the
		presence of flavonoids.
Detection of	A very small quantity of water and shell	A deep blue-black color
Tannins and	extract were combined, and then heated in a	(precipitates) appears in
Phenolic	water bath. After heating, the mixture was	addition to the 5% FeCI ₃
compounds (FeCI ₃	filtered and the filtrate mixed with ferric	solution.
Solution Test)	chloride.	
Detection of	About 1 mL of shell extracts was added to	The mixture formed stable
Saponins	20mL distilled water in a measuring cylinder	foam which indicates the
	and shaken for 15 minutes.	presence of saponins.
Test for	A measure of 5 mg of the extracts from each	The formation of pink color
anthraquinone	shell was boiled with about 10% HCl for	indicates the presence of
glycosides	2 mins in a water bath and the solution was	anthraquinones.
	filtered. Then the filtrate was allowed to	
	cool. Few drops of 10% NH ₃ and equal	
	volume of CHCL ₃ were added to the filtrate.	

Table 1. Preliminary Phytochemical Studies

Quantitative Phytochemical Analysis: Estimation of alkaloid: Prior to the absorbance test, 1 gram of *Cajanus cajan* and *vigna subterranea* shell samples were macerated in 20% con. H_2SO_4 for about 3 hours each, and the mixture was filtered. Then, 1 ml of the filtrate was mixed with 5 ml of 60% H₂SO₄, and the mixture was allowed to stand for an additional 3 hours. To find the absorbance, the solution was scanned at 490 nm. Three sets of the test were run, and the mean plus standard deviation of the data was presented. Regression analysis using an atropine standard curve produced an equation for calculating the total alkaloid content.

Estimation of Total Saponin Determination: The extract's saponin content was ascertained using the Vanillin–Sulfuric acid assay, as reported by Le et al.

(2018). This experiment involved mixing 5.0 ml of 72% (w/v) sulfuric acid, 0.5 ml of the sample solution, and 0.5 ml of an 8% (w/v) vanillin solution in an ice bath. After warming the mixture for ten minutes at 60°C in a water bath, it was chilled in ice-cold water. At 527 nm, absorbance measurements were made. For precision, each measurement was made three times.

Estimation of Tannin Content: The Van-Burden and Robinson (1981) method was modified slightly to determine the tannin concentration. First, 1 mL of the shell extract solution (0.1g/10 mL) was added to a test tube along with deionized water. This was mixed with 1 mL of 0.008 M K3Fe (CN) ⁶ (potassium ferricyanide) and 2 mL of 0.5 M FeCl₃ in 0.1 N HCl. After that, the mixture's absorbance was measured in ten minutes at 530 nm. The tannin concentration was expressed in

milligrams of tannic acid equivalents (TA) per gram of extract, with tannic acid serving as the reference standard for measurement. This quantification was accomplished using the regression equation that was derived from the standard curve.

Estimation of Steroids: To test the amount of steroids in the shell extract solution, 1 mL was carefully measured and added to 10 mL volumetric flasks. Next, 2 mL of 4N sulfuric acid and 2 mL of 0.5% w/v iron (III) chloride were added to the same solution. Then, 0.5 mL of a 0.5% w/v potassium hexacyanoferrate (III) solution was added. Subsequently, the mixture was heated in a water bath maintained at 70±2°C for 30 minutes, with periodic shaking Distilled water was added to the volumetric flask after heating in order to dilute the solution to the proper concentration. The absorbance of the solution was measured at 780 nm in comparison to a reagent blank. The regression equation that emerged from the cholesterol standard calibration curve was utilized to ascertain the steroid content. The steroid content that was expressed was mg/g cholesterol.

Estimation of Flavonoid Content: The total flavonoid content was measured using the aluminum chloride colorimetric method described by Zhishen *et al.* (1999) with a few minor modifications. 0.1 mL of the extract, 3.9 mL of distilled water, and 0.3 mL of 5% NaNO2 were put to a test tube. After five minutes, add 0.3 mL of 10% AlCl3, and after five more minutes, add 2 mL of 1 M NaOH. The solution was then increased to a volume of 10 milliliters by adding distilled water. Measurements of absorbance were taken at 510 nm against a blank after 15 minutes.

Estimation of Total Phenolic: A total phenol content analysis was performed on the plant extracts using the Folin-Ciocalteu technique. To be more exact, 3.9 mL of distilled water, 0.5 mL of Folin's reagent, and 0.1 mL of the extract were mixed together. We allowed the resulting combination to sit at room temperature for ten minutes. Following this period of incubation, 2 mL of a 25% sodium carbonate solution was added, and the test tube was then immersed in a boiling water bath for one minute.

The absorbance of the solution was measured at 650 nm. These measurements were carried out in triplicate, and the results were presented as Mean \pm Standard deviation A standard curve for gallic acid was utilized to determine the overall phenolic content. The regression equation produced from the standard curve was then used to calculate the total phenolic content, which was expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of extract.

Estimation of Cyanogenic Glycosides and Terpenoids: To find the cyanide concentration, 4 mL of an alkaline picrate solution and 1 mL of the filtrate were pipetted into a test tube. Subsequently, the combination spent five minutes being incubated at 90°C in a water bath. The absorbance of the solution at 490 nm was measured after the test tube had been allowed to cool to room temperature. A standard regression equation was utilized to ascertain the cyanide concentration in the sample, and this procedure was performed in triplicate. 0.1 g of the material were macerated with 20 mL of 80% ethanol and filtered as part of an additional step. The filtrate was then moved to a test tube, and 0.2 mL of it was combined with 1 mL of 10% phosphomolybdic acid. One milliliter of concentrated H2SO4 was then added. The 700 nm result was obtained thirty minutes after the ingredients had been well mixed in the test tubes. The total terpenoid content was computed using the Linalool calibration curve. With an R2 value of 0.9985, the data were expressed as Linalool equivalent (mg/g) using the usual plot regression equation, $Y = 0.0098x + 0.0144$.

RESULTS AND DISCUSSION

The GC-MS compositional studies of the chemical buildup of the extracts: The GC-MS chromatogram illustrated the major compounds detected in the shell extracts of C. *cajan* and V. *subterranean*, as shown in Fig. 1 and 2. Tables 2 and 3 present the major and active compounds, along with their concentrations. Fourteen chemical constituents were identified in both shell extracts.

From the GC-MS data, it was revealed that 4-allyl-2- 6dimethyl benzene, 4-vinyl guaiacol, Eugenol, 2 hydroxymethyl furan, isoeugenol, vinyl phenol, and levoglucosan are the predominant constituents in the extracts. Meanwhile, methyl phenol, 4-methyl guaiacol, vanillin, phenol, dimethylphenol, 2 furaldehyde, and dimethylbenzene are present in amounts below five percent each. These compounds, aside from their inhibitory effects, find applications in various domains, as listed in Table 4.

They can be utilized in industries for purposes such as food preservation, antioxidants, anti-inflammatory, antimicrobial, antibacterial, and anti-proliferative activities. Furthermore, they have implications in medicine and pharmaceuticals for drug development and treatments. Tables 2 and 3 indicate that phenolic compounds constitute about 50% of the chemical compositions of all the constituents in the shell extracts. This substantial percentage of phenolic content underscores its potential as an inhibitor of scale in industrial applications. This aligns with

previous studies that have reported similar findings (Abd-El-Khalek *et al*., 2016; Abdel-Gaber *et al*., 2008, 2011; Aidoud *et al*., 2017; Hajizadeh *et al*., 2019; Kadiri *et al*., 2018; Khaled, Abdel-Gaber, *et al*., 2020b; Wang *et al.*, 2016). Specifically, the GC-MS analysis of *C. cajan* indicates concentrations of inhibiting agents such as phenols (3.23 mg/kg), methyl phenol (4.87 mg/kg), dimethylphenol (2.92 mg/kg), vinylphenol (7.14 mg/kg), and 4-allyl-2- 6dimethoxphenol (14.61 mg/kg). Similarly, *V. subterranean* shows concentrations of phenols (3.99 mg/kg), methylphenol (4.49 mg/kg), dimethylphenol (3.66 mg/kg) , vinylphenol (11.64 mg/kg) , and 4 -allyl-2-6dimethoxphenol (8.31 mg/kg), all are active ingredients that aid inhibition.

FT-IR results of the C. cajan and V. Subterranean shell extract: The FT-IR analysis of both shells is depicted in Fig. 4, revealing the presence of alcohols, ketones, alkenes, alkanes, carbohydrates, and aromatics. These results align with the GC-MS findings, confirming the presence of active chemical ingredients in *C. cajan* and *V. subterranea* shell extracts. This showcases the potential of these shells to be deployed as scaling inhibitors. Upon comparing the results of GC-MS and FT-IR analyses, it becomes evident that the active chemical ingredients present in

the shell extracts are primarily phenolic and foaming compounds.

These compounds are known to be effective calcium carbonate scale inhibiting agents, further highlighting the suitability of *C. cajan* and *V. subterranean* shells for such applications. Furthermore, the FT-IR data confirms the presence of phenolics through the O-H stretching vibration at wave numbers of 3438 cm*-1* for *V. subterranean* and 3430 cm-1 for *C. cajan*. The other active compounds include the C=O band and C-O band at their respective wave numbers as shown in figure 4.

Phytochemical studies: The preliminary studies of phytochemicals, as outlined in Table 5, demonstrate that the shell extracts contain natural compounds capable of precipitating scaling-forming salts.

In the table, the presence of a compound is indicated by $(+)$, while its absence is denoted by $(-)$. The studies reveal the presence of seven (7) compounds, with phenolics, saponins, and tannins identified as the major inhibiting agents, all of which are present in the extracts.

Fig.1: Typical chromatogram of the compounds present in *C. cajan* shell extract.

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Table 2: the major and active compounds and concentrations of *C. Cajan* (sample K)

S/N Pesticides Maximum Conc. limit (mg/kg) Alpha-Value tvalue Pvalue Standard Deviation 1. **Phenol** 3.99 0.05 2.37 0.074 2.05 2. **Methylphenol** 4.49 0.05 4.02 0.042 0.25
3. **Dimethylphenol** 3.66 0.05 2.30 0.02 2.10 3. **Dimethylphenol** 3.66 0.05 2.30 0.02 2.10 4. **2-furaldehyde** 3.32 0.05 2.15 0.004 0.20 2hydroxymethylfuran 6. **Levoglucosan** 5.32 0.05 2.12 0.050 0.10 7. **4methylguaiacol** 13.30 0.05 3.20 0.05 2.30 8. **Vinylphenol** 11.64 0.05 1.19 0.060 1.55
9. **4-vinylguaiacol** 10.64 0.05 2.20 0.045 1.20 9. **4-vinylguaiacol** 10. **Eugenol** 10. **Eugenol** 14.96 0.05 2.10 0.04 0.25 11. **Vanillin** 5.98 0.05 1.80 0.004 1.10
12. Isoeugenol 5.91 0.05 2.15 0.064 0.20 12. Isoeugenol 5.91 0.05 2.15 0.064
4-allyl-2-6dimethoxphenol 8.31 0.05 3.25 0.052 13. 4-allyl-2-6dimethoxphenol 8.31 0.05 3.25 0.052 1.12 14. Dimethylbenzene 3.32 0.05 1.10 0.05 0.10

Table 3: The major and active compounds, their concentrations, of *V.Subterranean* (sample B)

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Quantitative phytochemical: **T**he quantitative phytochemical studies serve to reconfirm the presence and concentration of inhibiting agents and other compounds within *C. cajan* and *V. subterranean* shells. Notably, the percent concentrations of phenolics (6,294.65±117.35; 6,400.11±65.22)

 $(mg/100 g)$, saponins $(2.53\pm0.15; 1.79\pm0.04)$ (mg/100) g), and tannins $(176.49 \pm 13.18; 58.18 \pm 1.12)$ (mg/100) g) in the shell extracts, accounting for almost 50% of the chemical constituents, suggest that these extracts can effectively replace commercial synthetic chemicals in the scale industry.

HCN = cyanogenic compounds.

Conclusion: The comprehensive analysis of C. cajan and V. subterranea extracts reveals the presence of scale-inhibiting agents such as phenolic and foaming compounds, along with tannins. The results from GC-MS, FT-IR, and phytochemical studies consistently show the higher concentration of these inhibitory compounds, which according to various reports, has the capability to inhibit scale formation in petroleum pipelines. The qualitative and quantitative phytochemical analyses further support these findings by identifying satisfactory amounts of inhibiting

agents such as phenolics, saponins, and tannins. Overall, the compositional study validates that the shell extracts of *C. cajan* and *V. subterranean* can be effectively utilized in the industry for scale management.

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