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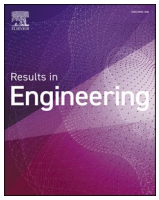

Phytochemical characterization of selected agro-waste extracts as kinetic inhibitors in methane hydrates formation

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ABSTRACT

In this study of gas hydrate inhibition, the bio-active components (phytochemicals) and the influence of functional moieties and chemical structures on selected bio-inhibitors' performance were investigated by advanced analytical techniques. The agro-waste materials of interest are: *orange mesocarp (Citrus sinensis (L)) extract* (OME), *red onion skin (Allium cepa) extract* (ROSE), *kolanut tesla (Cola acuminata) extract* (KTE), *coconut coir dusk (Cocos nucifera) extract* (CCDE) *and peanut skin (Arachis hypogea) extract* (PSE). A CO_2-enhanced supercritical fluid extraction (SFE) technique was used to extract the bioactive components from their parent materials. After the extraction, advanced analytical techniques including FTIR, NMR and HP-TLC with densitometry were used to identify and isolate functional groups, structurally characterize and quantify the bioactive compounds in the extracts primarily focusing on phenolic acids, flavonoids and tannins. A Bruker 500MZ NMR spectrometer and a CAMAG-semi-automatic HP-TLC system were used for the structural characterization and quantification respectively. Based on the number of anti-oxidants and radicals scavenging characteristics of the individual extracts' polyphenolic and tannin components, the expected hydrates inhibition capacities of the bio-extracts are in the magnitude: CCDE > ROSE > PSE > OME > KTE. The extracts are readily biodegradable and non-toxic and therefore do not pose ecological threat unlike PVP with only 31.50% biodegradation rate and may result in serious ecological risks in offshore environments. Lastly, the numbers of hydroxyl (OH) and meth-oxyl (MeoH) groups are the principal factors that influence the bio-inhibitors performance with hydroxyl group number being more important than that of the meth-oxyl group.

1. Introduction

The global demand for energy resources from fossil fuels is expected to increase by more than 50 % by the year 2030. To meet this demand, the flow of hydrocarbon fluids from the well sites through the flowlines to the sales points must be carefully monitored, most importantly in offshore environments, such as deep-water fields with unfavourable flow conditions (low temperature and high pressure). Therefore, effective flow assurance management practices that will prevent solid particle or hydrate deposition in pipelines must be implemented. This is necessary to forestall the economic, technical, operational, environmental and safety hazards that may result from blockage of flowlines due to hydrate or solid deposition. Gas hydrates are serious flow assurance concerns because they impede the flow of hydrocarbons in pipelines and processing facilities and may even constitute danger and safety concerns to personnel, thus representing a global barrier to the successful production of deep-water resources [1].

Gas hydrates are crystalline ice-like compounds in which water molecules (host) form a network of water cages which are held together by hydrogen bonding that encapsulates the guest molecules to form a molecular cage structure [2,3]. In this case, a large amount of natural gas liquid is trapped within the water crystal structure, forming an ice-like solid. These guest molecules (methane, ethane, propane, or their mixtures) are capable of residing within water cages. The water molecules form a lattice around the gas molecules to form a cage-like solid structure, which is referred to as a clathrate [4]. Four conditions are necessary for hydrate formation. These include high pressure as low as 2 MPa and low temperature of approximately 277 K, availability of hydrate formers or guest molecules (methane or ethane), presence of

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List of abbreviations

AAs	Anti-agglomerants (AAs)
AMD-2	Automated multiple development chamber
BB	Broad band
CCDE	Coconut coir dusk extract
DMAEMA	2- (Di-methyl amino)-ethyl methacrylate
DPBA	Diphenyl-boric-acid-2-amino-ethyl -ester
DSM	Densitometry
Equiv	Equivalent
ETs	Ellagitannins
FTIR	Fourier transform infra-red spectroscopy
GAE	Gallic acid equivalent
GTs	Gallo-tannins
HPLC pump	High-performance liquid chromatography pump
HP-TLC	High performance thin layer chromatography
IPMA	Isopropyl-meth-acrylamide
KHIs	Kinetic hydrate inhibitors
KTE	Kolanut tesla extract
LDHIs	Low dosage hydrate inhibitors (LDHIs)
MeOH	Methanol
MEG	Mono-ethylene glycol (MEG)
ND	Not detectable
OECD	Organization for economic cooperation and development
OME	Orange mesocarp extract
OPA	o-phthal-aldehyde
PLE	Pressurized liquid extraction
PSE	Peanut skin extract
PVcap	Poly-vinyl-caprolactam
PVP	Poly-vinyl-pyrrolidone
QE	Quercetin equivalent
ROSE	Red onion skin extract
SC-CO_2	Super critical carbon IV oxide
SFE	Super critical fluid extraction
TAE	Tannic acid equivalent
TBO	Triple resonance broad band probe
TFC	Total flavonoid content
THIs	Thermodynamic hydrate inhibitors
ThOD	Theoretical Oxygen demand
TLC	Thin-layer chromatography
TPC	Total phenolic content
UV-VIS	Ultraviolet–Visible Spectroscopy

List of symbols

+	Moderately Present
++	Present in higher quantities
+++	Strongly present (most abundant)

Chemical Equations

Pb $(C_2H_3O_2)_2$	lead II ethanoate solution
$CHCl_3$	Trichloromethane (chloroform)
$FeCl_3$	Iron III chloride solution
NH_4OH	Ammonia solution
H_2SO_4	Tetra-oxo-sulphate VI acid

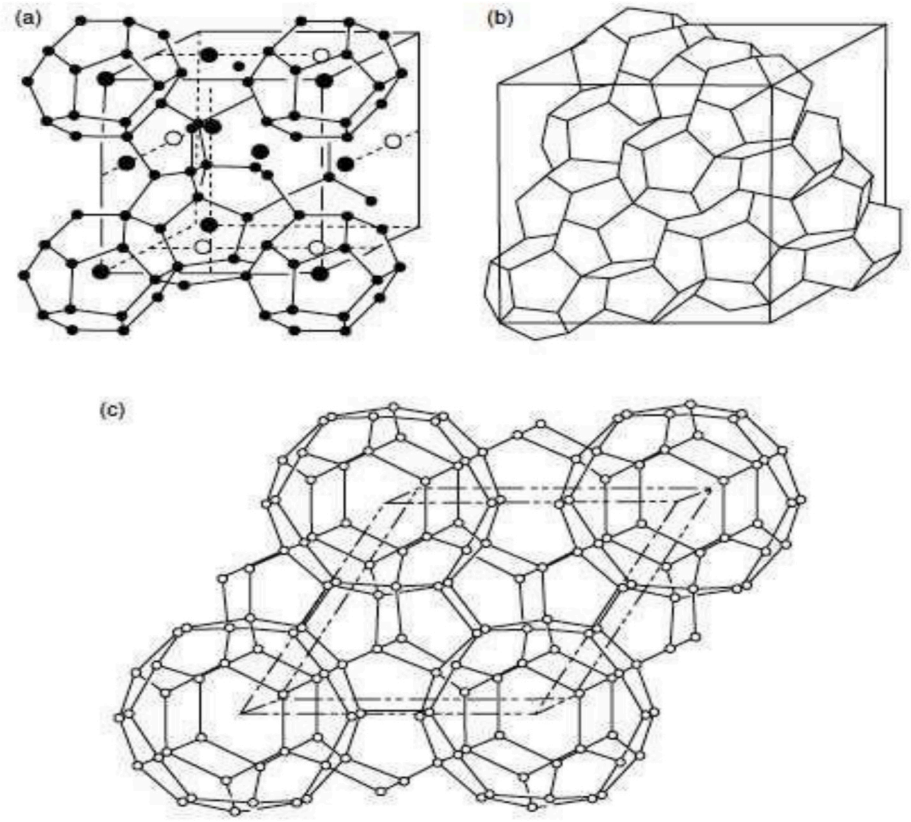


Fig. 1. Hydrate crystals (a) sI type, (b) sII type, (c) sH type [36–38].

sufficient free water quantities (host), and sufficient formation time. In oil and gas production operations, hydrates are usually formed whenever free water is available, and the temperature decreases below the hydrate formation in the flow string or surface line, or when there is a sudden pressure drop from expansion as the fluid flows through the orifice meter, chokes, or back pressure regulators. Agglomeration and deposition of these hydrates can occur on the walls of the flowlines during fluid flow or shut-in. Hydrates are usually identified based on their crystal structures. Based on this, we have cubic structure I (sI), cubic structure II (sII) and hexagonal structure (sH) as shown in Fig. 1.

Inhibition of gas hydrate formation can be achieved using conventional thermodynamic hydrate inhibitors (THIs) of which methanol (MeOH) and mono-ethylene glycol (MEG) are the most commonly used chemicals in this category. These inhibitors are introduced into the flowlines to maintain the fluid pressure and temperature outside the hydrate formation region. Thermodynamic hydrates inhibitors alter the hydrate-liquid-vapor equilibrium by establishing more stringent thermodynamic criteria for hydrate formation [5]. Methanol has a lower molecular weight and is easily vaporized into the gas phase for transportation in flowlines and plugs. Mono-ethylene glycol (MEG) is a preferred thermodynamic inhibitor because of the high flammability and toxicity of methanol, as well as its tendency for catalyst poisoning in the downstream unit.

Additionally, MEG is easily recoverable because it is injected into the flowline in the liquid state [6]. Low-dosage hydrate inhibitors (LDHIs), such as kinetic hydrate inhibitors (KHIs) and anti-agglomerants (AAs), are the second category of chemicals for hydrate inhibition. Anti-agglomerants (AAs) are large polymer-bridging agents that are mainly bio-surfactants, zwitterionic surfactants, phosphonium and ammonium salts in which one end of the molecule is joined to the hydrate structure and the other is dissolved in the oil phase.

KHIs are water-soluble polymers that dissolve in the carrier solvent and are injected into the liquid-water phase of the flowline. These inhibitors bond to the surface of hydrates and forestall tremendous crystal nucleation and growth for a time duration that is longer than the residence time of free water (host molecules) in a pipeline. Examples of KHIs are the Vinyl-lactam polymers, such as polyvinylpyrrolidone (PVP) and Poly-vinyl-caprolactam (PVCap). The others are isopropyl-methacrylamide (IPMA) and 2- (Di-methyl amino)-ethyl methacrylate (DMAEMA). These KHIs are applied to flowlines at exceptionally low concentrations. Specifically, a lower KHIs dosage, between 0.3 and 1.0 % by mass (3000–10,000 ppm), is sufficient for effective inhibition process [7]. According to Kelland *et al.* (2006) [8], KHIs concentrations ranging between 0.1 and 1.0 % (1000–10000 ppm) effectively retard hydrate formation.

The production of energy in the world must be carried out in a manner that promotes environmental and economic sustainability of the process (Abbasi *et al.*, 2022 [9], Abbasi and Erdebilli 2023 [10], Abbasi *et al.*, 2024 [11].

Currently, KHIs in the field are synthetic compounds that require sophisticated formulation techniques in terms of cost and raw material preparation. In addition, there are serious concerns about their toxicity and biodegradability because environmental regulatory and protection agencies require any chemical hydrate inhibitor to show at least 50 % biodegradation within 28 days before such chemicals can be applied in North Sea fields [12]. Current synthetic kinetic hydrate inhibitors are hazardous polymeric-chemical substances and these organic compounds are known to have consequential adverse effects on the aquatic lives and thereby creating serious ecological disturbances in the marine environment [13–18]. Degradation of these large persistent organic compounds requires the application of advanced technologies such as green nanotechnology, advanced materials and composites, and nanostructures for environmental remediation. These recent technologies involve highly sophisticated reaction mechanisms and pathways such as the application of nanocomposites as novel catalysts [19–21]. Similarly, complex ligand such as $[Cu(phen)_2(OAc)]$ PF_6 is usually employed as a photo-catalyst to degrade these high-molecular weight hazardous organic compounds [22]. Therefore, plant bio-materials help us in renewable and sustainable clean energy production, and storage as well as addressing environmental issues, sustainability and improving people's lives.

Hence, there is an urgent need to source for biodegradable and non-toxic chemicals from bio-materials that can provide similar or superior inhibitive capacity such as synthetic KHIs, while upholding economic and environmental advantages.

In recent years, some plant materials have been shown to possess various phytochemical substances in abundant quantities that can inhibit the formation and growth of hydrates [23–29]. Plant extracts contain abundant bioactive substances with significant anti-oxidant characteristics. These bioactive compounds are known as phytochemicals. These compounds include alkaloids, phenolic acids, flavonoids, steroids, terpenoids, isoprenoids, tannins, glycosides, saponins and quinones.

Flavonoids are hydroxylated polyphenolic compounds that are mostly found in fruits, bark, and stems. They are produced by the phenyl-propanol synthetic pathway and are 2-phenyl benzyl-pyrone derivatives [30]. It is a fifteen-carbon atom containing two benzene rings that are linked together by three-carbon chains and subdivided into flavones, flavonols, flavanones, flavan-3-ol, and anthocyanins based on their chemical structure arrangement, oxidation extent, and linking chain unsaturation. It is a 15-carbon atom consisting of two phenyl rings and a heterocyclic ring that contains an attached oxygen.

Phenolic acids (PAs) are naturally occurring polyphenols. They are predominantly aromatic acids that contain carboxyl acid groups and phenolic rings. They are ubiquitous in various plants. It includes organic acids with phenolic rings and carboxylic acid functional group with C_1–C_6 carbon skeleton [31]. These acids can be vanillic, *p*-coumaric, chlorogenic, caffeic, or ferulic acids. Sali-cyclic acid is one of the most naturally abundant phenolic acids.

On the other hand, alkaloids are naturally occurring organic compounds with at least one nitrogen molecule in their hydrocarbon chain. It may also contain oxygen in addition to the hydrocarbons and nitrogen [32]. They are secondary metabolites that are ubiquitous in plants and are grouped based on hydrocarbon-nitrogen structural relationships, such as pyrrolidone, quinoline, piperidine, and iso-quinoline. Terpenoids are naturally abundant organic compounds obtained from five-carbon isoprene units. They are also called isoprenoids and are terpene derivatives of oxygen molecules. All terpenes have five-carbon repeating isoprene units. This compound is known to possess appreciable anti-oxidant activity. Terpenes are hydrocarbons with 10–15 carbon atoms, whereas terpenoids are modified terpenes in which the methyl moieties are shifted or replaced when oxygen atoms are added to the hydrocarbon chain [33–35]. Paclitaxel (Taxol) and menthol are the two most prominent terpenoids. Tannins are polyphenolic compounds containing significant hydroxyl and carboxyl moieties. They are widely distributed throughout plants. Saponins are triterpene glycoside derivatives that occur naturally in plants and soap-bark trees. Other phytochemicals include quinones, steroids, and glycosides.

The practical application of this research work is the use of phenolic compounds and other phytochemicals that are present in some agro-waste extracts as kinetic hydrates inhibitors to prevent gas hydrates nucleation and growth in flowlines/pipelines during oil and gas transportation in offshore environment.

Finally, the novelty of this work is that the authors employed advanced analytical techniques including SFE, FTIR, NMR, HP-TLC and WIN CATS version 1.4.4.6337 software to isolate, characterize and quantify bioactive compounds in the agro-waste materials primarily focusing on phenolics, flavonoids and tannins. The results highlight the rich polyphenolic content and anti-oxidant capacity of red onion skin, coconut coir dusk and peanut skin extracts, suggesting their promise as eco-friendly kinetic hydrate inhibitors.

The objectives of this present study are highlighted below. These are:

i. Extraction of the bioactive components of the selected agro-waste biomass by a CO_2-enhanced supercritical fluid extraction (SFE) technique.
ii. Qualitative phytochemical screening and isolation of phenolic compounds and other phytochemicals from the plant extracts.
iii. Application of advanced analytical techniques such as FTIR, HNMR, HP-TLC and WIN CATS version 1.4.4.6337 software to isolate, characterize and quantify bioactive compounds.
iv. Establishment of the mechanisms of kinetic hydrates inhibition by the bioactive components.
v. Application of nuclear magnetic resonance to know the influence of functional moieties and structure on inhibitors' performance.
vi. Assessment of the environmental acceptability of the local inhibitors through biodegradability test and comparison with a known synthetic kinetic hydrate inhibitor named Poly-vinyl-pyrrolidone (PVP).

The remaining sections of this research study would be focusing on the screening of selected agro-waste materials and the application of super-critical fluid extraction technology to remove the bioactive components from their parent materials using super-critical carbon IV oxide. The SFE process is inevitable where high product purity is paramount because, unlike other extraction processes where some residual solvent is left in the extract and matrix with some level of environmental contamination, the process leaves no residue. Moreover, CO_2 is easily removed by reducing the pressure without leaving any residue. After the extraction, a preliminary phytochemical screening and isolation was carried out by modified standard phytochemical analysis in order to test for flavonoids, phenolic acid, alkaloids, glycosides, tannins, saponins, terpenoids and quinones. This was followed by the application of advanced analytical techniques such as FTIR, HNMR, HP-TLC AND WIN CATS version 1.4.4.6337 software to isolate, characterize and quantify the isolated bioactive compounds. The next section involves the establishment of the hydrate's inhibition mechanisms by the bioactive components through the prevention of radical's formation owing to their strong anti-oxidant's characteristics and the consequent delay of crystals nucleation and growth. Moreover, nuclear magnetic resonance (NMR) was used to investigate the molecular structure of the extracts at the atomic level and these NMR spectra were used to assess the influence of functional groups and chemical structure on inhibitors' performance. Finally, the aerobic biodegradation of the different inhibitor samples was evaluated by using Oxygen (O_2) consumption manometric-respirometry procedures as highlighted by OECD guidelines. This test was used to evaluate the environmental friendliness of the bio-inhibitors in offshore environments.

2. Materials and methods

2.1. Selected agro-waste bio-materials

The following agro-waste materials were selected for this study. These are:

1. Orange mesocarp (*Citrus sinensis (L)*)
2. Red onion skin (*Allium cepa*)
3. Kolanut tesla (*Cola acuminata*)
4. Coconut coir dusk (*Cocos nucifera*)
5. Peanut skin (*Arachis hypogea*)

2.2. Production of plant extracts from selected agro-waste bio-materials by supercritical fluid extraction (SFE) technology

This process involves the separation of bioactive components from their parent materials (matrix) using a supercritical fluid as an extraction solvent. The two most commonly employed supercritical fluids are water (H_2O) and carbon IV oxide (CO_2). However, CO_2 is the most technically and economically viable supercritical fluid owing to its operational advantages [39–41].

These are:

i. Colourless, odourless, non-inflammable and non-toxic gas
ii. Readily availability and lower cost
iii. Safe and environmentally friendly with unambiguous critical operating parameters (critical temperature of 31.2 °C and critical pressure of 74 bar).
iv. The critical temperature is very low to support the extraction of thermally unstable compounds.
v. Pure and can be recycled

Under supercritical fluid conditions, CO_2 gas shows the characteristics of a lipophilic solvent (lipid-soluble compounds) with an intermediate behavior between gas and liquid. Supercritical CO_2 has low polarity characteristics with increased suitability for the dissolution of fats and oil, but has deficiencies in dissolving polar compounds. Hence, other co-solvents, such as t-butanol, methanol, ethanol, and water, are used to increase the polarity tendency [42–44]. That is, the co-solvents promoted the solubilizing capacity of SC-CO_2 for the extraction of more polar compounds. An HPLC pump is often used to add these co-solvents to the SC-CO_2.

2.2.1. Laboratory Procedures

The SFE apparatus consists of a reciprocating CO_2 pump, cylinder, reaction vessel to house the sample, pressure maintaining device, inlet and outlet valves, heating jacket, vent valve, cooler, pre-heater coil, and collecting vessel. Fig. 2 shows the schematic representation of the SFE process.

Different plant-biomass specimens were cleaned and separated using sieves. Thereafter, they were washed and dried in a microwave oven at 60 °C for 3 h. Dried powdery bio-materials were then weighed by a calibrated weighing balance and stored under low temperature condition to form a molten matrix before they were transferred into the extractor vessel of the SFE apparatus that is shown in Fig. 2 and all the outlet and inlet valves were closed immediately. The extraction stages were matrix penetration, solubilization of the solutes by the supercritical solvent (CO_2) inside the pores of the plant materials, diffusion of the solute from the internal to the external surface, external diffusion of the solutes from the solid-fluid interface of the supercritical CO_2 and solute precipitation by modifying the reaction temperature and pressure until a suitable pressure was reached.

Carbon (IV) oxide modified with ethanol as the co-solvent was used as the extraction solvent. Ethanol was used as a co-solvent owing to its intrinsic characteristics of forming hydrogen bonds with solutes and enhancing the supercritical CO_2 density, resulting in higher miscibility and solubility of the solvent in the solutes [45]. The CO_2 was pumped in liquid form at 41 °F and a pressure of 50 bar into an oven, where it was heated to a supercritical temperature of 60 °C. At an oven temperature of 60 °C and pump pressure of 50 bar, the CO_2 inlet and outlet valves were opened to allow SC-CO_2 flow into the extractor vessel, where it quickly diffused into the solid matrix and dissolved the bio-materials. The solubilized bio-materials were washed away from the reaction vessel into a separator at a lower pressure to allow the extracts to settle. Thereafter, extracts were collected in a vessel by opening the outlet and exit valves. CO_2 was then cooled, recompressed, and discharged into the atmosphere. The extracts were then filtered, concentrated to dryness under low pressure using a rotary vacuum evaporator, lyophilized to remove traces of water molecules, and stored at 20 °C for subsequent characterization and analysis.

2.3. Preliminary qualitative phytochemicals screening of agro-waste extracts

The procedures highlighted by standard phytochemical analysis

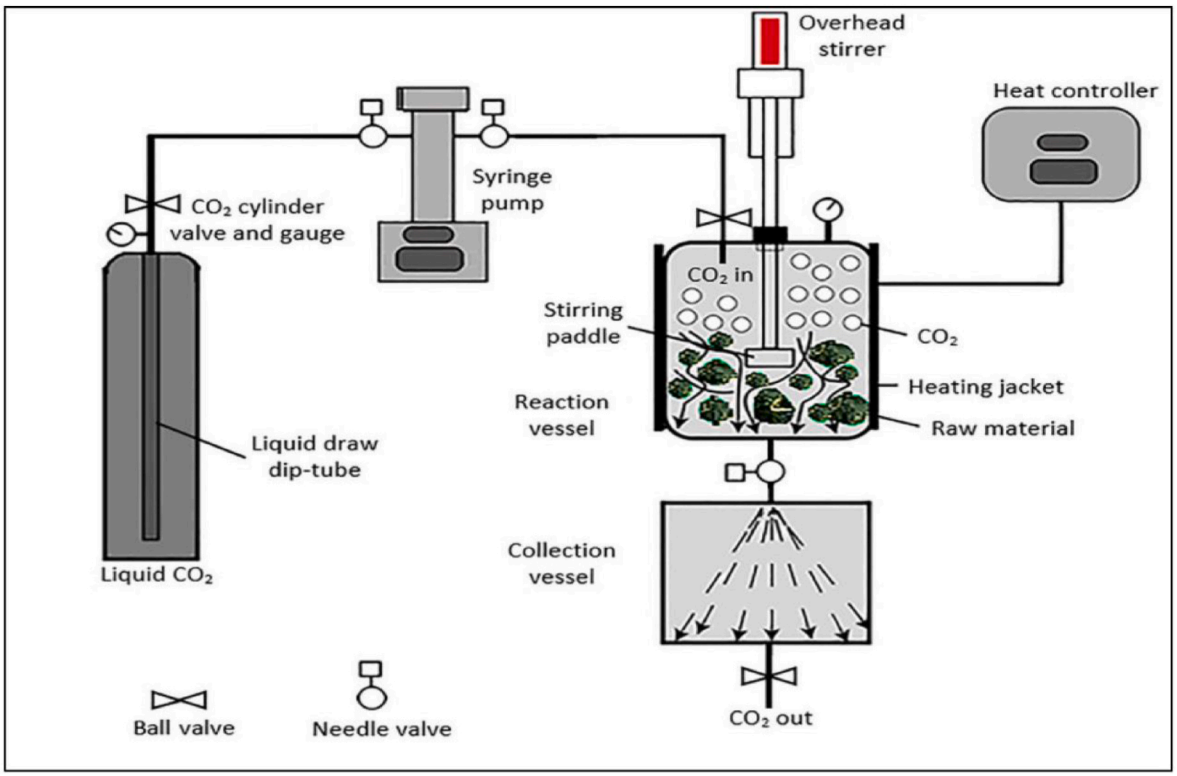


Fig. 2. Supercritical Fluid Extraction Process [46].

were modified and used in the preliminary phytochemical screening of each plant extract [47].

(I) Test for Flavonoids

Approximately three drops of sodium hydroxide solution (NaOH) were added to 3 ml of each plant extract. A deep yellow color was observed. The solution became colorless upon the addition of two drops of dilute hydrochloric acid (HCl). This indicates the presence of flavonoids. The test was confirmed by adding 5 ml of dilute ammonia solution to the aqueous filtrate of each extract. This was followed by the addition of a concentrated H_2SO_4 solution. A yellow color which varnished on standing was observed. This confirmed the presence of flavonoids.

(II) Test for Phenolic Compounds

Approximately a few drops of 5 % aqueous solution of iron (III) chloride ($FeCl_3$) were reacted with 50 mg of each plant extract which had been dissolved in 5 ml of distilled water. A dark-green color was observed, indicating the presence of phenolic acid.

(III) Test for Alkaloids

The presence of alkaloids in each agro-waste extract was investigated by using the Mayer's reagent. These results were confirmed by using Wagner's reagent. In Mayer's test, approximately two drops of the reagent were added to 5 ml of each plant extract in a test tube, and the appearance of a white creamy precipitate was indicative of the presence of alkaloids. However, the addition of a few drops of Wagner's solution (2 g iodine + 6 g potassium iodide in 100 ml of distilled water) to the plant extract resulted in the formation of a brown precipitate, which served as a confirmatory test for the presence of alkaloids.

(IV) Test for Glycosides

The availability of glycosides in a plant extract can be determined by hydrolyzing approximately 50 mg of the extract with concentrated hydrochloric acid (HCl) for a period of 90-min in a water bath. The resulting mixture was then filtered. At the end of the process, approximately 2.5 ml of trichloromethane ($CHCl_3$, Chloroform) were added to 2 mL of the filtered hydrolysate in a test tube, and the resulting mixture was shaken adequately and left to settle. The trichloromethane layer was then separated, and approximately 10 % ammonia solution (NH_4OH) was added. The formation of pink color is indicative of the presence of glycosides. This test is referred to as Borntrager's test.

(V) Test for Tannins

Each extract was diluted with distilled water at the ratio of 1:4 and three drops of 10 % iron III chloride ($FeCl_3$) solution were added. The solution turned green, which indicated the presence of tannins. The test was confirmed by weighing 1 g of powdered samples in a flask and adding approximately 10 ml of distilled water to form a homogeneous solution. The solution was then heated to 75 °C for 5 min. Subsequently, two drops of a 5 % iron III chloride ($FeCl_3$) solution were added. A green precipitate was formed, confirming the presence of tannins.

(VI) Test for Terpenoids

Approximately 5 ml of each extract was mixed with 3 ml of trichloromethane ($CHCl_3$) (chloroform) in a test vessel. This was followed by the addition of 3 ml of conc H_2SO_4 resulting in the formation of a reddish-brown layer.

(VII) Test for Saponins

The presence of saponin was tested by the addition of 1 % lead II ethanoate solution ($Pb(C_2H_3O_2)_2$ to one milli-litre of each plant extract. A white precipitate was formed, confirming the presence of saponin.

(VIII) Test for Quinones

The presence of quinones was confirmed by the addition of a drop of concentrated H_2SO_4 to approximately 10 mg of each plant extract which was initially dissolved in isopropyl alcohol. Only red onion skin showed positive results with the formation of a red color solution.

2.4. Spectroscopic analysis of the agro-waste extracts by (FTIR) technique

The identification of functional moieties present in the individual agro-waste extract was carried out in the laboratory using an Agilent 5500t FTIR spectrometer with a TUMBIIR sampling interface and a micro-lab computer accessory. This equipment has sophisticated mechanisms for oil analysis in offshore fields.

Laboratory Procedure.

1. The TUMBIIR sampling interface was thoroughly cleaned and the sample collection background was carefully removed.
2. The FTIR machine was connected to its personal micro-lab computer.
3. Approximately 2 μL of each plant extract were introduced into the FTIR system by opening the TUMBIIR sampling interface, and its arm was then rotated in an anti-clockwise direction. The power button was then switched on for analysis and turned off when it was completed.

Note: The PH of each plant extract was measured before FTIR analysis to prevent the corrosion of the FTIR plate.

2.5. Isolation of phenolic compounds and other phytochemicals from the plant extracts

(a) Isolation of Flavonoids

Flavonoids were extracted from the plant materials by maceration using 70 % ethanol in water as the extraction solvent. The process was conducted in the laboratory by soaking the biomaterials in an appropriate solvent for a period of 72-h at room temperature. The synthesized phytochemical mixtures were filtered and transferred to a column chromatography apparatus to isolate flavonoids. However, maceration requires large amount of solvent, longer process duration, and requires further purification [48]. Polar flavonoids such as anthocyanins, flavonols, flavonones, and flavones, were extracted using the pressurized hot water technique at 110 °C. Careful control of the extraction temperature and process duration is highly important in hot water extraction because the destruction of flavonoids may occur from longer exposure periods and at temperatures higher than 120 °C. The process parameters were: a temperature of 120 °C, extraction duration of 15-min and a solute-to-solvent ratio of 1:34.

The pressurized liquid extraction (PLE) yield is influenced by temperature, pressure, time, nature of the biomaterial, and solute-to-solvent ratio.

(b) Isolation of Phenolic Acids

A non-polar acetone solvent was used to extract the phenolics from each of the designated bio-materials. The bio-materials were crushed and the fats were removed by dissolving 5 g of each plant material in 50 ml of hexane solvent in a vessel. After fat removal, the homogeneous solution was mixed with 50 ml of acetone and heated to 55 °C for 45min to extract phenolic compounds. The extract was centrifuged at 30 °C. Vacuum evaporation at 65 °C was used to remove the solvent, and the filtrate was lyophilized at 40 °C for 24hrs to remove any present water.

(c) Isolation of Alkaloids

The alkaloids were isolated from their parent bio-materials by mechanically grinding the materials in a pulverizer mill, followed by sieving of the materials and drying at 100 °C. The dried bio-materials were then treated with alkali, followed by extraction with methanol in a Soxhlet apparatus. The pressure was reduced to evaporate the solvent and synthesize a methanol-extract that was fractionated. Alkaloids were isolated from plant crude extracts by solvent extraction, followed by liquid chromatography using a silica-gel stationary phase.

However, for other phytochemicals, approximately 0.5 g of dried sample of the bio-materials was dissolved in a methanol mixture and agitated for 30 min and passed through a 0.45 mm pore size syringe filter before being injected into the HP-TLC system.

2.6. Characterization of the isolated compounds by nuclear magnetic resonance

Nuclear magnetic resonance spectroscopy (NMR) provides comprehensive information concerning the functional moieties, molecular structures, reaction states, and chemical surroundings of the molecules. The fundamental principle of the NMR is based on the existence of atomic nuclei in the nuclear spin states when there is an interaction with an external magnetic field. The Bruker 500MZ spectrometer (Fig. 3) has a sample autosampler that could accommodate 24-samples. The installed probe was a 5 mm smart probe with a broad-band and ^{1}H channels. There was also a 5 mm TRP (Triple resonance probe) with broad-band (BB), ^{1}H, and ^{19}F channels. The machine was operated at upper temperature limit of 150 °C.

2.6.1. Laboratory Procedures

Approximately 50 mg of each isolated phytochemical compound was dissolved in 750 μL of water at room temperature in a nuclear magnetic resonance (NMR) tube to make a solution in order to obtain high-resolution NMR spectra. The solubilized sample was injected into the NMR machine using a sample Case autosampler. The operating temperature was set at 115 °C. The mechanism of NMR spectroscopy in the sequential order is described below. The first stage is the magnetic nuclear spin alignment or polarization in a given constant magnetic field. These aligned or polarized nuclear spins are perturbed by a weak oscillating magnetic field (radio-frequency pulse). Finally, the perturbation induces the sample nuclei to emit electromagnetic waves that are detected and analyzed using a spectrometer.

Fig. 3. Nuclear magnetic resonance spectrometer (Bruker Avance III, 500 MHZ).

2.7. Qualitative and quantitative characterization of the isolated phytochemicals

2.7.1. Phenolic compounds and other bioactive components characterization

The isolated phytochemicals were characterized by using a high-performance thin-layer chromatography with densitometry (HP-TLC with densitometry).

High-performance thin-layer chromatography (HPTLC) analysis: instrumentation and operating conditions.

The HPTLC test was carried out in the laboratory by using a CAMAG HPTLC system (Fig. 4) which is a semi-automated system. The system consists of an auto sampler that supports automatic sample application by changing the syringe only, a multiple development chamber that is fully automated, and constitutes the most important part of the HPTLC system that controls stationary phase activities. The AMD-2 chamber enhances the thin layer chromatography analysis of reproducible gradient elution when silica gel is employed as the stationary phase. The HPTLC apparatus also consists of a derivatization chamber that contains an appropriate derivatization agent to enhance the detection of the separated components, which are colorless and cannot be picked with ultra-violet light and fluorescence. These reagents were applied to the TLC stationary plate, and upon reaction with the samples, they were converted to detectable derivatives. The derivatization agent for flavonoid was diphenyl-boric-acid-2-amino-ethyl-ester (DPBA). The TLC plate could also be derivatized using a mixture of boric acid (3 %) and oxalic acid (10 %) for flavonoid separation. This reagent was used to improve flavonoid fluorescence because flavonoids have been tagged to exhibit poor fluorescence characteristics [49]. Acyl chloride was used as the derivatization agent for terpenoid analysis because the major sites for terpenoid derivatization are –OH and –COOH. However, the most common derivatization agents are o-phthal-aldehyde (OPA), Benzoyl Chloride and Dansyl chloride, with a greater focus on OPA owing to its short duration, because derivatization can be completed within less than 2-min in the presence of a buffer solution (borate) with a PH of 6–8 and methanol under ambient conditions. CAMAG Reprostar 3 was used for documentation, whereas Scanner 3 was used for detection and analysis.

2.7.2. Laboratory Procedures

Equal volumes of the test samples and standard solution (phenols) (2.5 μL) were applied to the stationary plate of the TLC with the aid of a syringe in the auto sampler at 8-mm band length. The TLC plate was pre-coated with silica gel 60 F_{254} with a thickness of 200 μm. TLC plates with already loaded samples were saturated with the respective solvent and vertically placed in a TLC twin-trough development chamber. The plates were then saturated at a temperature of 20–25 °C for 30mins using the recommended mobile phases. The following mobile phases were used in the experiment according to Wagner *et al.*, 1984 [50]. These are:

i. Standard solution (Phenol): Toluene-acetone-formic acid (4.5:4.5:1) v/v/v
ii. Flavonoids: Ethyl acetate: water: formic acid: acetic acid (100:26:11:11) v/v/v/v
iii. Phenolic acids: Toluene: ethyl acetate (93:7)
iv. Alkaloids: Toluene: ethyl acetate: diethyl amine (70:20:10) v/v/v
v. Saponins: Chloroform: glacial acetic acid: methanol: water (6.4:3.2:1.2:0.8) v/v/v/v
vi. Glycosides: Ethyl acetate-ethanol-water (8:2:1.2) v/v/v
vii. Tannin: Toluene-ethyl acetate-formic acid-methanol (3:3:0.8:0.2) v/v/v/v
viii. Terpenoids: Hexane: ethyl acetate: methanol (8.2:1.8:0.5) v/v/v
ix. Quinones: Petroleum ether: Ethyl acetate (7:3)

In addition, appropriate spraying reagents, as recommended by Kogje *et al.* (2010) [51] and Sounder and Doss (2016) [52] for each isolated phytochemical, were also applied. Folin-Ciocalteu solution with ammonia vapor was used as a spraying agent for flavonoids, phenols, and phenolic acids; 5 % ferric chloride solution was used for tannins; Liebermann Burchard reagent (10 % H_2SO_4 in ethanol +10 % acetic anhydride) was used for terpenoids and glycosides; 10 % ethanolic KOH solution was used for Quinones while Dragendoff solution with 10 % ethanolic sulfuric acid was used as the spraying agent for alkaloids. After saturation with the recommended mobile phases, the TLC plate was developed to a height of 80 mm, in ascending order from the bottom. Hot air was then passed through the surface of the developed plate to dry the plate by removing any solvent via evaporation from the mobile phase.

The dried and developed TLC plate was then sprayed with the appropriate spraying reagents and dipped into the recommended derivatizing agents for approximately 10s in the derivatization chamber to promote TLC separation performance and sensitivity. The plate was dried with hot air and heated at 100 °C in a thin-layer chromatography (TLC) oven for 15 min and the hot plate was kept in a Reprostar 3 documentation chamber. It was then fixed to an automatic TLC scanner, where scanning was performed using UV-light with a wavelength of 254 nm. During scanning, careful attention was given to the peak table, peak display, and peak densitogram. A UV-densitometer was used for chromatogram quantification at different wavelengths. The bands were quantified by densitometry using a CAMAG TLC scanner 3 with the aid of WINCATS version 1.4.4.6337 software. The scanner operated in absorption-reflection mode with a scanning rate of 20 mm/s and a bandwidth of 20 nm for monochromatic wavelengths of 245 and 366 nm. The process was monitored using WINCATS (version 1.4.4.6337) software.

Fig. 4. CAMAG semi-automatic HPTLC system.

3. Results and discussion

3.1. Percentage yield and product purity of the extracts by supercritical technology

The average percentage yield of the extracts was 97.50 %, with approximately 98.80 % purity. The conventional method of soxhlet extraction, where a small amount of dried parent material in a soxhlet extractor is reacted with a non-polar solvent such as hexane, has been characterized with various process ambiguities. These include: large solvent and energy consumption, longer process duration owing to poor mass transfer rates, lower solvent recovery, and non-suitability for thermally unstable compounds. This makes the process less efficient [53,54]. Supercritical fluid extraction (SFE) on the other hand, is a fast, reliable, highly efficient, economical and solvent-free extraction method of extracting bioactive materials from plant biomass [55–57]. The process involves a small amount of solvent, such as alcohol and water, and does not form any solvent residue because the different phases are

separated by depressurization. In the supercritical fluid extraction process, a component referred to as the extractant is separated from another one known as the matrix with the aid of an extraction solvent called a supercritical fluid.

The various parameters that influence the efficiency of the extraction process are solvent flow rate, diameter of the extracting vessel or reactor diameter, nature and size of parent materials because larger particles require more time for extraction, and vice-versa, reactor diameter-to-length ratio, bed porosity, and stirring diameter [58]. Therefore, different temperatures, pressures, and solvent flow rates are usually combined in numerous reactor series to enhance the selectivity and sequential separation of bioactive substances from plant bio-materials [59].

3.2. Spectroscopic analysis of the agro-waste extracts by (FTIR) technique

FTIR spectra are used for the detection or identification of different functional groups in organic and inorganic compounds. The FTIR spectra of all agro-waste extracts are shown in Fig. 5a–e. As shown in Fig. 5a (FTIR spectrum of orange mesocarp extract), a symmetrical methyl stretch was observed at 2926 cm^{-1} suggesting that the compound contained a substantial amount of methyl ester components [60–62]. In addition, the group frequency of 1710 cm^{-1} is suggestive of the presence of a carboxylic acid compound. A bend with a group frequency of 1025 cm^{-1} is indicative of the presence of a bi-cyclo carboxylic acid compound. There is a direct attachment of a hydroxyl group to a diene compound to form a di-enol, as indicated by several inward bends with group frequencies 864-767 cm^{-1}.

The FTIR spectrum of the red onion skin extract (A*llium cepa*) is shown in Fig. 5b. Based on this spectrum, there is a significant presence of carboxylic acid at the group frequency in the range of 1725–1700 cm^{-1} precisely at 1707 cm^{-1}. In addition, there is a direct attachment of a hydroxyl group to a diene compound to form a di-enol, as indicated by several inward bends with a group frequency of 816.3 cm^{-1}.

Fig. 5c shows the FTIR spectrum of the kolanut tesla extract (*Cola acuminata*). There was a significant presence of carboxylic acid at a group frequency of 1699.7 cm^{-1} with great occurrence of *cis*-fatty acid presence with different carbon stretches at 1602 cm^{-1}. Similarly, a symmetrical methyl stretch was observed at 2926 cm^{-1} suggesting that the compound contained a substantial amount of methyl ester compounds. Some traces of caffeine groups (stretching vibrations of carbonyl group within the wavelength range of 1650–1750 cm^{-1}) were observed in the FTIR spectrum of the kolanut tesla extract (Fig. 5c).

However, a symmetrical carbon stretch occurs at 1606 cm^{-1}, 1517 cm^{-1} and 1438 cm^{-1} in the FTIR spectrum of the coconut coir dusk extract (*Cocos nucifera*) (Fig. 5d). This indicates the presence of carboxylic acids.

Finally, from the FTIR spectrum of peanut skin extract (*Arachis hypogea*) in Fig. 5e–a C–O vibration occurs at 1010 cm^{-1} with a broad OH vibration that occurs at a group frequency of 3288 cm^{-1} and an OH- out-of-plane vibration that occurs at 872.2 cm^{-1} which is suggestive of the presence of a tertiary alcohol. Out of plane broad band vibrations occur due to stretching vibrations of bonded and non-bonded -O-H groups. Furthermore, the symmetrical carbon stretches at 1606 cm^{-1}, 1520 cm^{-1} and 1442.5 cm^{-1} is indicative of the presence of carboxylic acid groups.

3.3. Qualitative and quantitative characterization of extracts

The results of the HPTLC quantitative and qualitative analyses are shown in Table 1a and b for the individual extract. HPTLC enables the detection, separation, and identification of bioactive phytochemicals in each agro-waste extract.

High-performance thin layer chromatography is a highly advanced, efficient, novel, automated, and accurate extension of thin layer chromatography with superior and higher separation efficiency and limits of detection [63]. Like normal TLC, HPTLC uses adsorption as the main separation technique, but with superior analytical precision and accuracy, high reliability, and can be suitably applied for confirmatory qualitative and quantitative analyses of different compounds [64,65]. It offers different advantages over conventional TLC, such as short duration, ease of compound separation, and flexibility [66].

The predominant alkaloids in the orange mesocarp extract were synephrine (a colorless alkaloid soluble in water) and octopamine (phenyl-ethanol-amines) (Table 1a). Each of the two alkaloids has one hydroxyl group. The orange mesocarp contains significant amounts of phenolic compounds, including quercetin, quercitrin, rutin, and catechin, which constitute the major flavonoid compounds, while ferulic, caffeic, and gallic acids are the detectable phenolic acids (Table 1a).

The red onion skin extract (*Allium cepa*) (Table 1a) contained the highest number of phenolic compounds. The amounts of flavonoids and phenolic acids in the extract are 78.16 ± 2.30 mgQE/g and 142.50 ± 1.7mgGAE/g respectively. The flavonoids detected were quercetin, anthocyanin, fisetin and quercetin-di glucose. Vanillic acid, gallic acid, protocatechuic acid, and its glucose constitute the major phenolic acids (Table 1a), with high amounts of theobromine and theophylline as detectable alkaloids. The kolanut tesla extract (Table 1a) had the lowest amounts of polyphenolic compounds, with small amounts of catechin and epicatechin as the main flavonoids (0.33 ± 0.002 mgQE/g) while the phenolic acids were chlorogenic, quinic, and tannic acids (21.40 ± 0.85mgGAE/g). Caffein was the primary alkaloid in the kolanut tesla extract (Table 1a). This invariably results in poor methane hydrate inhibition.

The coconut coir dusk extract has the highest amount of hydrolysable and non-hydrolysable tannins (tannoids), with a small quantity of natural polyphenols. The total tannin content in the extract is 141.5 ± 5.08 mgTAE/g with gallotannins (GTs) and ellagitannins (ETs) representing the major hydrolysable tannins in the extract while pro-anthocyanidins and phloba-tannins are the present non-hydrolysable or condensed tannins in the coconut coir dusk extract (Table 1b). This results in superior methane hydrates inhibition capacities. The peanut skin extract is very rich in polyphenols most especially in phenolic acid with 3.54 ± 0.15mgQE/g and 78.61 ± 2.02 mgGAE/g flavonoids and phenolic acids respectively (Table 1b). The extract also contained a significant amount of hydroxyl and methoxyl propanoic acid alkaloids. However, quinones were not detected in any of the plant extract samples except in red onion skin where about 0.90 g of anthra-quinones were detected. These phytochemicals can act as anti-oxidants, structural polymers (lignin), attractants (flavonoids and carotenoids), UV screens (flavonoids), signal compounds (sali-cyclic acid and flavonoids), and defense response chemicals (tannins and phytoalexins) [67].

Phenolic compounds (flavonoids and phenolic acids), tannins, and alkaloids are the most important bioactive components in plant materials that initiate kinetic hydrate inhibition.

In comparison with previous researchers (Table 2), Omoba *et al.* 2015 [68] employed a high-performance liquid chromatography (HPLC) technique with diode array detector (DAD) to analyze the bioactive components in orange mesocarp. The authors concluded that the orange mesocarp extract has a total flavonoid (TFC) of 4.20 mgQE/g and a phenolic content (TPC) of 9.40mgGAE/g with quercitrin, rutin, quercetin, catechin and caffeic acid as the major phenolic components [69, 70].

The total flavonoid content (TFC) in red onion skin according to different investigators was found to be 20.22 ± 0.39mgQE/g [71] and 49.69 ± 0.55mgQE/g [72]. While the total phenolic compounds (TPC) ranges from 63.62 ± 2.03 to 208.42 ± 4.24 (mgGAE/g) [73]. Nile *et al.*, 2021 [74] identified the major phenolics in red onion skin extract as quercetin,3,4 and 7,4-di glucose, vallinic acid and protocatechuic glucose and its acid. Also, the major phytochemicals in Kolanut tesla extract as identified by Atawodi *et al.*, 2007 [75] are catechin, caffeine, epicatechin and procyanidin (Table 2). The amounts of total flavonoids

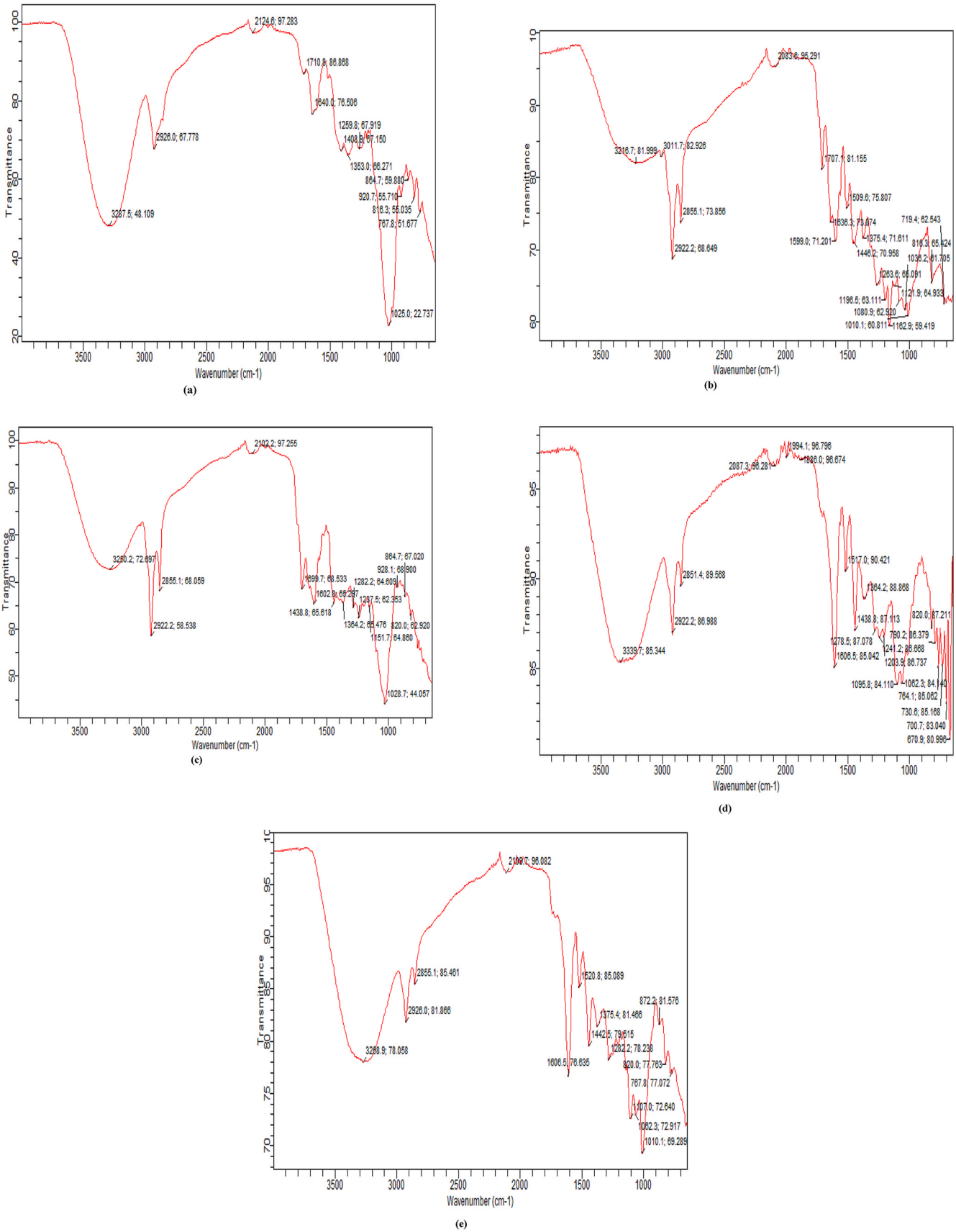


Fig. 5. FTIR spectrum: Orange mesocarp extract (a), red onion skin extract (b), kolanut tesla extract (c), coconut coir dusk extract (d), peanut skin extract (e). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1a
HP-TLC of Orange Mesocarp Extract (OME) (*Citrus sinensis (L)*), Red Onion Skin Extract (ROSE) (*Allium cepa*) and kolanut Tesla Extract (KTE) (*Cola acuminata*).

	ALKALOIDS	FLAVONOIDS	PHENOLIC ACIDS	TARPENOIDS	TANNINS (TANNOIDS)	SAPONINS	QUINONES
(1) OME Present/ Absent	+	+	++	+	+ (low)	+	ND
Amount	1.65 ± 0.05 mg/g	3.71 ± 0.15 mgQE/g	25.61 ± 1.72 mgGAE/g	1.48 ± 0.10 Mg/g iso-prene-equiv	0.26 ± 0.001 mgTAE/g	0.74 ± 0.006 Mg/ g	N/A
Major Phyto-chemical	Synephrine Octopamine	Quercetin Quercitrin Rutin Catechin	1 Ferulic acid 2 Caffeic acid 3Gallicacid	Limonene Myrcene	Gallo- Tannins	Glycyrr-Hizin (glycosides)	ND
(2) ROSE (Present/ Absent)	+	++	+++	+	+	+	+
Amount	3.18 ± 0.16 mg/g	78.16 ± 2.30 mgQE/g	142.50 ± 1.7 mgGAE/g	2.75 ± 0.12 Mg/g iso-prene-equ	1.50 ± 0.1 mgTAE/g	1.16 ± 0.05 Mg/g	0.94 ± 0.01 mg/g
Major Phyto-chemical	Theobromine Theophyline	Quercetin^{++} Anthocyanin Fisetin Quercetin-di glucose	1Vanillic acid. 2Protocate-Chuic acid 3 PCA-lucose 4Gallic-acid	Copaenes (a-tricyclic sesquiter penes)	Gallo-Tannins	(glycosides)	Anthra-quinones
(3) KTE Present/ Absent	+	+	++	trace	+	+	ND
Amount	0.98 ± 0.0075 mg/ g	0.33 ± 0.002 mgQE/g	21.40 ± 0.85 mgGAE/g	–	0.81 ± 0.005 mgTAE/g	0.51 ± 0.003 Mg/ g	N/A
Major Phyto-chemical	Caffein^{++} Teo- bromine	Catechin Epicatechin	Chloro-genic acid Quinic-acid Tannic-acid	–	Phloba-phene	(glucosides)	ND
Method of Analysis	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM

Table 1b
HP-TLC of coconut coir dusk extract (CCDE) (*Cocos nucifera*) and peanut skin extract (PSE) (*Arachis hypogea*).

	ALKALOIDS	FLAVONOIDS	PHENOLIC ACID	TARPE-NOIDS	TANNINS (TANNOIDS)	SAPONINS	QUINONES
(4) CCDE Present/ Absent	+	+	++	–	+++	Trace	ND
Amount	1.71 ± 0.051 mg/g	1.20 ± 0.022 mgQE/g	32.15 ± 0.96 mgGAE/g	–	141.5 ± 5.08 mgTAE/g		N/A
Major Phyto-chemical	Atropine+	Catechin Epicatechin Procyanidins (dimer & polymer	Gallic acid Elagic acid Chloro-genic acid Protocate-chuic acid Vanillic-acid	–	1 Proanth ocyanidins 2 gallo-tannins 3 Ellagi-tannins 4 Phloba-tannins	(glucosides)	ND
(5)PSE Present/ Absent	+	+	+++	trace	+	+ (low)	ND
Amount	3.12 ± 0.11 mg/g	3.54 ± 0.15 mgQE/g	78.61 ± 2.02 mgGAE/g	trace	0.70 ± 0.005 mgTAE/g	0.36 ± 0.003 Mg/g	N/A
Major Phyto-chemical	2-methoxyl-3-(3-indolyl) propionic acid 2-hydroxyl-3-[3-(1-N-methyl-indolyl] propionic acid	Quercetin Diosmetin Diosmetin	1 Caftaric Acid^{+++} 2 trans-Coutaric acid^{++} 3Cis-coutaric acid 4 Proto-catechuic acid 5 Hydroxyl Benzoic acid	ND	Phloba-Tannins	(glycosides)	ND
Method of Analysis	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM	HP-TLC With DSM

+ = Moderately Present; ++ = present in higher quantity; +++ = strongly present (most abundant); GAE = Gallic Acid equivalent; QE = Quercetin equivalent; TAE = Tannic acid equivalent; DSM = densitometry; HP-TLC = high-performance thin layer chromatography; Equiv. = Equivalent, ND = Not detectable.

and phenolics in coconut tesla have been previously quantified by Dah-Nouvlessounon *et al.*, 2015 [76] as 5.37 ± 0.92(mgQE/g) and 24.44 ± 0.81(mgGAE/g) respectively. Additionally, Karseno *et al.*, 2023 [77] employed a Folin-coicalteu calorimetry technique to conclude that the coconut coir dusk has a TFC value of 1.57 mgQE/g and a phenolic content (TPC) of 76.04 mgGAE/g. Meanwhile, a reversed-phase high-performance liquid chromatography was used by Chuenchom *et al.*, 2016 [78] to characterize the phenolic compounds in peanut skin extract. The TFC was found to be 75.77 ± 14.33 mgQE/g while the TPC was 154.56 ± 29.39 mgGAE/g with p-coumaric acid as the most abundant bio-active compound.

There are close proximities between the values of quantified

Table 2
Results of bio-active components characterization from previous studies.

Agro-waste biomass	Method of analysis	Total Flavonoid (TFC) (mgQE/g)	Total Phenolic (TPC) (mgGAE/g)	Major Bioactive components	References
Orange Mesocarp Extract	HPLC-DAD	4.20	9.40	Quercitrin Rutin Quercetin Catechin Caffeic acid	[68] [69] [70]
Red onion Skin Extract	HPLC/MS	20.22 ± 0.39 49.69 ± 0.55	63.62 ± 2.03 to 208.42 ± 4.24	Quercetin Vanillic acid Protocatechuic acid	[71] [72] [73] [74]
Kola nut Tesla Extract	HPLC-ESI-MS	5.37 ± 0.92	24.44 ± 0.81	Catechin Caffeine Epicatechin Procyanidin	[75] [76]
Coconut Coir Dusk extract	Folin-coicalteu calorimetry/UV-VIS	1.57	76.04	Tannin (522.95mgTAE/g)	[77]
Peanut Skin Extract	RP-HPLC	75.77 ± 14.33	154.56 ± 29.39	p-coumaric acid p-hydroxybenzoic Vanillic acid Ferulic acid	[78]

phytochemicals in this study and those of previous studies. However, the little variations may not be unconnected with the types of plant materials cultivars (genetics), environmental factors, cultivation conditions, sample preparation technique, method of extraction employed, extract purity as well as the quantification technique that was used.

3.4. Mechanisms of kinetic hydrates inhibition by phytochemicals

When these agro-waste extracts are introduced into the flowlines, the phytochemicals that are present in them, mostly flavonoids, phenolic acids, tannins, and saponins, have strong anti-oxidant properties that prevent radical formation. These soluble anti-oxidants in the locally formulated kinetic hydrate inhibitors would delay the nucleation of hydrate crystals and thus progressively hindering crystal growth after nucleation. With the development of hydrates, the alkyl groups in agro-waste extracts invade the cavity where the liquid hydrocarbon resides. The carbonyl group of the amide would also penetrate the cavity to prevent hydrate nucleation and growth.

However, during this chemical process, the inhibitors do not have any significant effect on the thermodynamic characteristics of hydrate formation because the chemical does not shift the relationship between the hydrate-liquid-vapor equilibrium (HLVE) [79]. These locally formulated kinetic inhibitors will delay the crystallization of hydrates for a period that is sufficient for flowing oil, gas, and water (hydrocarbons and associated water) to exit production lines prior to plugging the line by hydrates [80,81]. These chemicals elongate the hydrate crystal nucleation period, thereby extending the crystal growth duration. These nucleation periods are defined by induction times. The effectiveness of these inhibitors is measured by the extent to which the inhibitors can counteract subcooling upon exposure to operating temperature and pressure conditions [82,83] because more subcooling results in higher rates of hydrate formation and lowers the hydrate induction period [84]. The first step in the KHIs inhibition process is the disruption of the molecular structure of hydrogen-bonded water in the vicinity of the evolving hydrate crystal [85]. The functional groups present in plant extracts can obstruct the nucleation of hydrate crystals by reducing the near-by-free water molecules and imminent water structuring [86–88]. The second phase involves the adsorption of inhibitor molecules on the surface of the embryo crystals. This mechanism hinders nucleation by preventing the diffusion of water or gas because adsorption creates crooked paths for diffusion and thus slows the movement of hydrocarbon gases to that surface [89,90]. Therefore, the adsorption of KHIs onto embryo crystal surfaces unarguably retards crystal growth. When the inhibitor binds to hydrates crystal surfaces, crystal growth is repressed leading to the development of a plate-like crystal [91]. The inhibitor forms a cluster around the crystal surfaces, and methane gas may be blocked in some cavities; however, some uncovered crystal faces are still accessible through the crooked path.

3.5. Influence of functional moieties and structure on inhibitors' performance by nuclear magnetic resonance

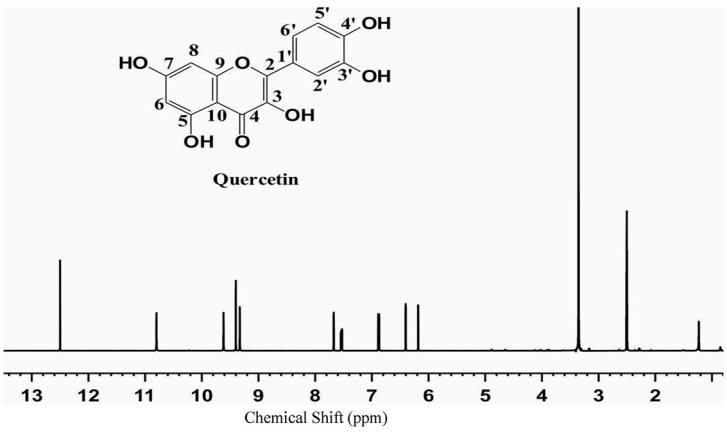
Nuclear magnetic resonance spectroscopy is primarily used to determine the molecular structure of a compound at the atomic level, alterations in molecular structure conformation and configuration, as well as solubility and diffusion potentials [92]. Fig. 6a–f represent the HNMR structures of the major phytochemicals in the agro-waste extracts.

The red onion skin extract (*Allium cepa*) will have significant methane hydrates crystals inhibition capacity when deployed in hydrocarbon flowlines because it contains the highest quantities of polyphenolic compounds with 78.16 ± 2.30mgQE/g and 142.50 ± 1.7mgGAE/g flavonoids and phenolic acids respectively. Phenolics are associated with high anti-oxidant capacity owing to their intrinsic ability to scavenge free radicals and active oxygen species, such as single oxygen, superoxide free radicals, and hydroxyl radicals [93]. These radical-scavenging activities are attributed to hydroxyl-group replacement in the aromatic ring of phenolic compounds owing to their ability to donate hydrogen [94].

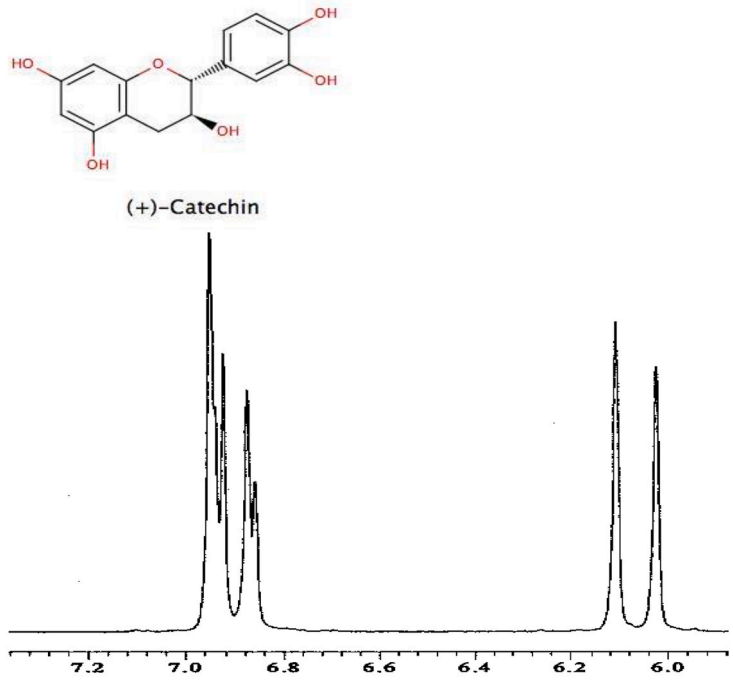
However, better hydrates inhibition potential would be observed with the coconut coir dusk extract (*Cocos nucifera*) because of its high tannins content (141.5 ± 5.08mgTAE/g) with gallotannins (GTs) and ellagitannins (ETs) constituting the major hydrolyzable tannin compounds. Tannins have a higher radical scavenging potential within a lower concentration range, but phenolic compound scavenging activity varies among different compounds [95]. Moreover, tannin radical scavenging activity is usually enhanced by galloyl group numbers, molecular weight, and the structure of ortho-hydroxyl groups, whereas phenolic compound scavenging capacity is influenced by the number and position of hydroxyl or meth-oxyl groups.

Phenolic acids have the intrinsic ability to neutralize free radicals to enhance their anti-oxidant tendencies. This unique characteristic can be linked to the acidic nature of their chemical structure, with hydroxyl moieties at different positions [96]. Phenolic acids can interact with each other to induce more anti-oxidant characteristics.

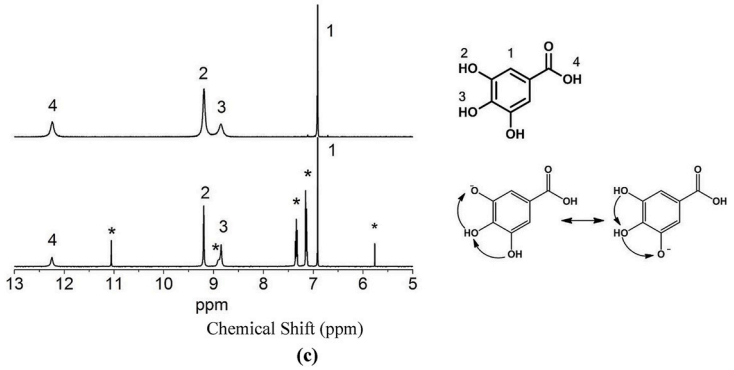
The primary factor that enhances the anti-oxidant potential of



(a)

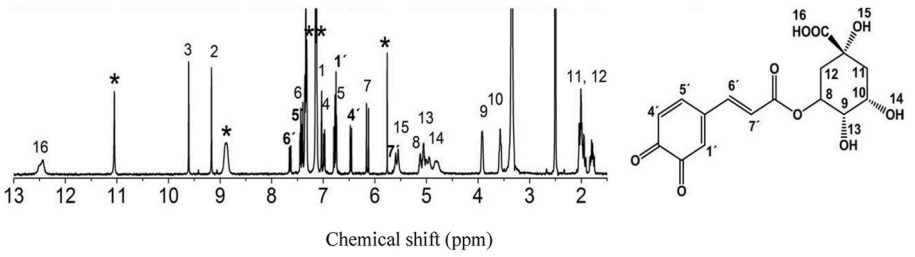


(b)

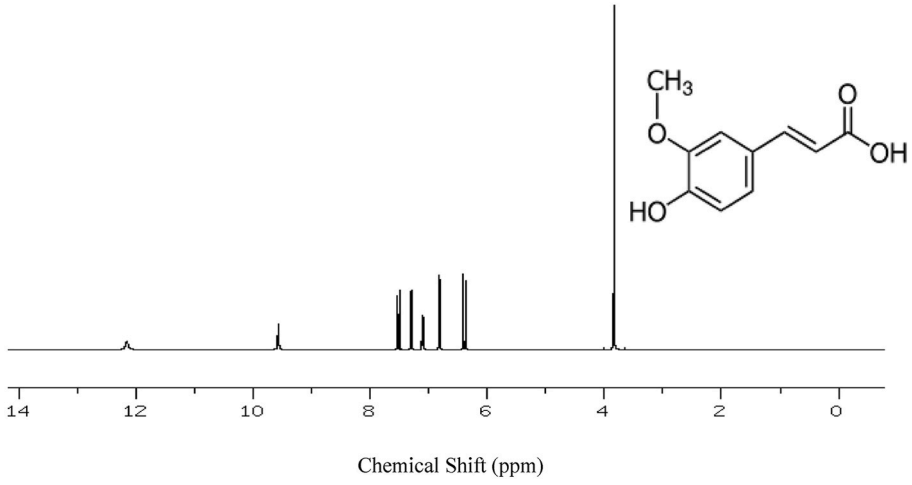


(c)

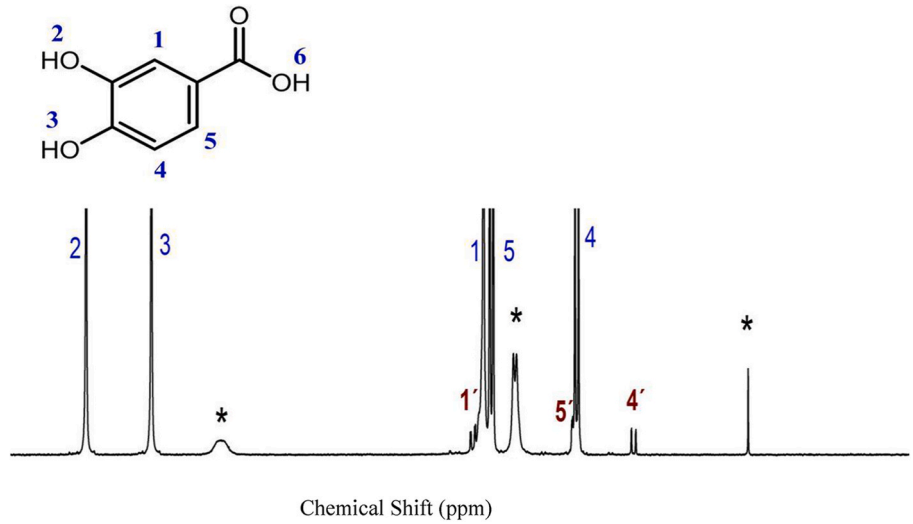
Fig. 6. HNMR Spectrum: Quercetin (a), Catechin (b), Gallic acid (c), Chlorogenic acid (d), Ferulic acid (e), Protocatechuic acid (f).



(d)



(e)



(f)

Fig. 6. (*continued*).

phenolic compounds is the nature of their chemical structures. It is imperative to state that the greater the number of hydroxyl (OH) and meth-oxyl (MeOH) groups, the higher the anti-oxidant activity [97–99]. The number of hydroxyl groups is greater than that of the meth-oxyl group. Based on the number of hydroxyl groups, the increasing order of anti-oxidant activities was as follows: caffeic acid was more active than ferulic acid, and ferulic acid was more active than *p*-coumaric acids.

3.6. Aerobic biodegradability of different inhibitor samples

The aerobic biodegradation of the different inhibitor samples was evaluated by using Oxygen (O_2) consumption manometric-respirometry procedures as highlighted by OECD guidelines for aerobic degradation in sea water [100–102]. All the agro-waste extracts are readily biodegradable as seen in Table 3 because aerobic degradation was more than 70 % at the end of twenty-eight days (28) incubation period. Similarly,

Table 3
Aerobic biodegradation of different inhibitors samples.

Time (Days)	OME	ROSE	KTE	CCDE	PSE	PVP	Reference
	ThOD = 48.90 mg/L	ThOD = 37.01 mg/L	ThOD = 46.86 mg/L	ThOD = 46.86 mg/L	ThOD = 26.61 mg/L	ThOD = 33.05 mg/L	Chemical ThOD = 78 mg/L
0	0	0	0	0	0	0	0
$N_1 = 5$	31.25	34.82	28.50	36.20	38.10	9.30	45.30
$N_2 = 10$	54.16	60.30	49.10	61.45	63.35	14.50	70.60
$N_3 = 15$	60.95	67.10	57.31	68.30	69.10	21.00	78.50
$N_4 = 20$	68.40	72.35	65.95	74.10	76.80	24.95	82.10
$N_5 = 28$	73.60	77.41	71.32	78.15	81.46	31.50	85.60

at the end of the 10-day window period, the biodegradation extents were more than 60 % except in OME and KTE agro-extracts where biodegradation extents were 54.16 % and 49.10 % respectively (Table 3). This implies that all the plant biomass extracts are inherently biodegradable except the OME and KTE (orange mesocarp and kolanut tesla extracts) [103]. This means that significant amount of oxygen was consumed by the aerobic microbes in the inoculum containing the different agro-waste extracts. However, the Poly-vinyl-pyrrolidone (PVP) synthetic hydrate inhibitor is non-biodegradable with only 31.50 % degradation extent at the end of the incubation period. This is due to the fact that an insignificant amount of oxygen was consumed compared to the theoretically available value. This supports the environmental friendliness of the agro-waste extracts over conventional synthetic inhibitors.

4. Conclusions

The supercritical fluid extraction (SFE) is a reliable, highly efficient, economical, and solvent-free method for extracting bioactive materials from plant biomass. These bio-extracts are non-toxic as shown in their FTIR profiles because they contain mainly of methyl ester functional groups with carboxylic acid moieties. Phytochemicals such as phenolic compounds, tannins and saponins are bio-degradable compounds with strong anti-oxidants power that prevents radicals' formation and therefore highly efficient as kinetic hydrates inhibitors. These soluble anti-oxidants delay nucleation of hydrates crystals and thus progressively hindering crystal growth after nucleation. In terms of structure-property relationship, the numbers of hydroxyl (OH) and meth-oxyl (MeoH) groups are the principal factors that influence bio-inhibitors performance. Finally, the agro-extracts are readily bio-degradable and do not constitute ecological threats unlike Poly-vinyl-pyrrolidone (PVP) with only 31.50 % biodegradation extent and may result in serious ecological risks in offshore environments. The expected hydrates inhibition capacities of the bio-extracts in terms of polyphenolic and tannin components are in the magnitude: CCDE > ROSE > PSE > OME > KTE.

5. Recommendations for further studies

1. Development of predictive structure-activity relationships (SAR) models in future work to aid the rational design and selection of new plant-based hydrate inhibitors
2. Advancement of these sustainable anti-hydrate agents towards field application and commercialization, scale-up considerations, formulation development and techno-economic analysis to guide future work.

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CRediT authorship contribution statement

Okon Efiong Okon: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Joseph Atubokiki Ajienka:** Writing – review & editing, Visualization, Supervision, Resources, Conceptualization. **Sunday Sunday Ikiensikimama:** Writing – review & editing, Visualization, Supervision, Resources, Conceptualization. **Onyewuchi Emmanuel Akaranta:** Writing – review & editing, Visualization, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this article. This paper represents the unanimous and genuine purpose of scientific interest.

Data availability

No data was used for the research described in the article.

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