INVESTIGATION ON POLYPHENOLIC COMPOUNDS DERIVED FROM TROPICAL HERBS FOR INHIBITING SILICATE SCALES

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CERTIFICATION OF APPROVAL

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by

Muhammad Taufiq Bin Ahmad

13194

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Approved by,

____
(Associate Professor Dr. Ismail Bin Mohd Saaid)

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CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

(MUHAMMAD TAUFIQ BIN AHMAD)

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ABSTRACT

Silicate scale deposition in region around the wellbore or in any parts of the production system is a serious production problem. During hydrofluoric acidizing treatments, the release of silicon and aluminum sets off a series of reactions that include hydrolysis of silicic acid, condensation and eventual polymerization of amorphous silica. These reactions negate the original aim of inhibiting silicate scales. Currently, most chemical inhibitors are harmful and not environmental friendly. Therefore, environmental friendly scale inhibitors extracted from plants are beginning to get attention as possible alternative. This paper presents experimental findings on tropical herbs such as *premna cordifolia, camellia sinensis, and piper betle* and *abelmoschus esculentus* for preventing silicate scales. Effects of polyphenolic compounds were examined in mitigating the polymerization and condensation of the silicic acid. The performance of selected herbs was evaluated at different concentrations and a range of pH before comparing them against the conventional green inhibitor, polyaspartate (PASP). Experimentally, 100 ml samples of brines representing formation water were added with 100 ppm, 500 ppm and 1000 ppm polyphenolic compounds from the four herbs respectively. Observation was made for 24 hours at $90^{o}C$ during the compatibility test and at interval of 1,2,3,4,5 and finally 24 hours for turbidity test at pH 1-5. The loss of transparency due to the presence of suspended particulate, colloidal material, or both represents the degree of turbidity. Results showed that green inhibitors at concentrations of 100 ppm, 500 ppm and 1000 ppm did not cause precipitation. Turbidity of all samples decreased when concentration of polyphenolic compounds increased. Camellia sinensis, due to its high content of polyphenolic compounds caused the least turbidite compared to the other green inhibitors. This finding justifies other published works where polyphenolic compounds had acted as a chelating agent in the form of silicon-polymerizing complexes to condense and polymerize silicic acid in brines to prevent formation of silicate scaling. Nevertheless, turbidity of all brines added with green inhibitors showed higher turbidity values than that of brines added with PASP. It might be attributed to the high concentration of chlorophyll since the extracts were used in their pure forms without other treatments.

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CHAPTER 1 : INTRODUCTION

Chapter one describes the background study, problem statement, objectives, scope of studies, project significance and relevancy.

1.1 Background Study.

Few production problems strike fear into the heart of oil and gas engineers the way scale can. Silica scale is an assemblage of deposits that cake perforation, casing, production tubing, valves, pumps and downhole completion equipment, thereby clogging the wellbore and preventing fluid flow. Scale inhibiting chemicals can be applied up or downhole of the wellhead and generally has been classified into four categories: Oil-Miscible, Totally Water Free, Emulsified and Solid. Depending on the mineral content present in the water, duration of the project and operation needs, the chemicals can be applied continuously or in scale squeeze applications (Kelland and Malcolm, 2009).

According to Jasbir *et al* (2011), the standard way to accomplish the characterization of silicate using an integrated approach involving polarized light microscopy, X-ray diffraction and SEM - EDS analysis. The terms silica and silicates are often used interchangeably. In fact, silica and silicate represent two distinct families of compounds. These terms should not be used interchangeably. Silica generally refers to $SiO_{2.}$ Silica dissolves in water to form a very weakly ionized species, silicic acid, which may be represented by the formula H_4SiO_4. Silica, in the solid phase as silicon dioxide, is identified in its crystalline form as quartz or in its non-crystalline form as amorphous silica. Amorphous refers to the lack of an ordered or crystalline structure determined by X-ray diffraction. The compounds cannot be identified by its characteristic crystallanity since the particle size is so minute. Meanwhile, silicate refers to the compounds of silicic acid, which are formed by reacting ionized silica with metals such as Ca, Mg, Al, Fe, Zn, etc. Clays, such as kaolinite and illite are examples of silicates found in brine as water-borne silica deposits (Jasbir and Gregory, 2011).

Several of these factors and processes may take place concurrently, making it difficult to predict equilibrium solubility and how scale formation by presence of silica. To prevent silica based deposit, it is essential to control all these paths, simultaneously. As engineers, are aware that silica scale is extremely tenacious, highly insulating, and very difficult to remove, fortunately there have been many significant advances in silicate scale control and remediation in recent years. These information comes from research of preventing silica fouling in industrial water system which can be apply to the oil and gas field production system.

The scale inhibitors can be divided into two categories which threshold inhibitor and non-threshold inhibitor (Arensdorf *et al,* 2011). The threshold inhibitor will totally prevent the formation of silicate scaling meanwhile non-threshold inhibitor will slow down the formation of scaling. Threshold inhibitor obviously better from non-threshold as it stops the scale from producing, but non-threshold is good enough as it delays the formation and gives enough time to drive the oil to the surface. As been practiced in oil and gas industry nowadays , the use of chemical to prevent or slow down the silicate scale formation are contained with toxic and contaminants, thus will indirectly affected the environment. Thus, studies must be extended further into finding silicate scale inhibitor which is more environmental friendly and non-toxic to the surroundings. The identified plants are *Premna cordifolia* (Bebuas), *Camellia sinensis* (Teh), Piper *Betle* (Sirih) and *Abelmoschus esculentus* (Kacang Bendi). These chosen plants have good potential to be green inhibitors besides having a lot of benefits in medical practice and abundant in Malaysia.

1.2 Problem Statement

Silica-based deposits are particularly difficult to remove once they formed. The precipitation of hydrated silica, $SiO_2(H_2O)_2$ has been a problem in sandstone acidizing and continues to be recurring in hydrocarbon zones. The industry might use some chemicals to overcome the secondary and tertiary reaction. After other chemical employed to overcome the consequence of post-HF acidizing treatment, the chemical directly discharge to surrounding by sipping through the land and sea.

The chemicals used in some ways are harmful and possible to bring negative impact to the environment, disturbing the equilibrium of ecosystem. The final effect will be unsafe, and environmentally unsustainable such the soil will be contaminated and give negative impact to the crops beside the animals or sea inhabitants which consume the plant which has absorb some contaminant or toxic. But in this research, it focusing the use of organic material from plants which had potential in silicate scale inhibition as silica polymerization inhibitor (SPI), a laboratory work and analysis of result had to be done.

1.3 Objectives

Governments and environmental groups becoming increasingly aware of the positive impact that can be brought on the environment by use of safe, non-toxic and green inhibitors. The objectives of this project are as follow:

- To extract the polyphenolic compounds from identified tropical herbs
- To analyze the identified plants which comprise of green inhibitor performance at different concentrations and pH.
- To compare the performance between green inhibitor and conventional green inhibitor.

1.4 Scope of Studies

The scopes of study in this project are :

- Conducting experiment to investigate the performance of inhibitor extracted from the identified plant and its potential to become alternative silicate scale inhibitor .
- Focusing to the effect after HF acidizing treatment on sandstone formation as there are possibility of secondary and tertiary reaction which can negate the original aims.

1.5 Project Significance and Relevancy

The green silicate scale inhibitor project is very significant because it is environmental friendly alternative for oil and gas industries in mitigation silicate scaling. By using natural occurring substance, the environments are not being polluted with the other chemicals effects if there is fluid circulation loss. This project research also relevant to be conducted as using natural plants which has low cost which can be produced commercially in lower price. The sources of this organic silicate scale are very wide as being produced everywhere that lead to unlimited resource in the future. Further studies need to be done to produce efficiently, effectively and commercially since oilfield industry still lack of environmentally friendly inhibitor for field implementation.

CHAPTER 2 : LITERATURE REVIEWS

Second chapter of the reports elaborates about the silicate scale formations, problems arising due to silicate scales, polyphenolic compounds as scales inhibitors and plants which has been identified with polyphenolic compounds.

2.1 Silicate Scale Formations

Formation of silicate scales is a physical-chemical process. It is controlled by the following factors (either respective or combination):

- amorphous silica solubility
- temperature
- composition
- pH of solution
- concentration of colloidal particles
- hydrodynamic conditions in brine flow
- precipitation with other multivalent ions example aluminum, iron
- biological activity in the water

Generally, as soon as a well begins to produce water, there is risk of inorganic scale formation. The severity of the problem will depend upon the water chemistry, the rate of drawdown, and other factors which have been mentioned above. Supersaturation may be caused by changing the temperature and pressure of a water or by mixing incompatible waters. Besides, scale problems may be encountered when new water injection wells are commissioned if the injection water is intrinsically incompatible with the formation water.

Furthermore, a mineral salt does not precipitate when its concentration in the solution reaches the saturation. The ions are still dispersed and an increase in concentration is required to allow the ions to collide and form a nucleus. Other ions and nuclei will agglomerate and nuclei will start precipitating, preferentially in crystal imperfections. Sufficient contact time between the supersaturated solution and surface is necessary to form scale. The longer the exposure time is, the greater the amount of crystal growth. Crystal growth is essential to form a scale. For example, a hard silica scale is formed when calcium carbonate or other mineral precipitate provides a crystalline matrix in which silica can be entrapped.

$$Si(OH)_4 + OH^- \longrightarrow (OH)_3SiO^- + H_2O$$

$$Si(OH)_3^- + Si(OH)_4 \longrightarrow \underset{\text{(dimer)}}{(OH)_3Si - O - Si(OH)_3} + OH^-$$

$$\text{Dimer} \longrightarrow \text{Cyclic} \longrightarrow \text{Colloidal} \longrightarrow \text{Amorphous Silica (scale)}$$

Figure 2.1 : Formation of amorphous silica

Silica scaling is the largest problem that displays practically at all geothermal fields, especially at the high-temperature, because fluid forms in contact with rocks where silicium is the basic rock-forming element. The solubility of silica is decreasing when temperature de-creases. As opposed to carbonate, silica deposition is controlled by kinetics and can begin on the surface in several minutes or hours after reaching supersaturation (Amjad and Zuhl, 2008).

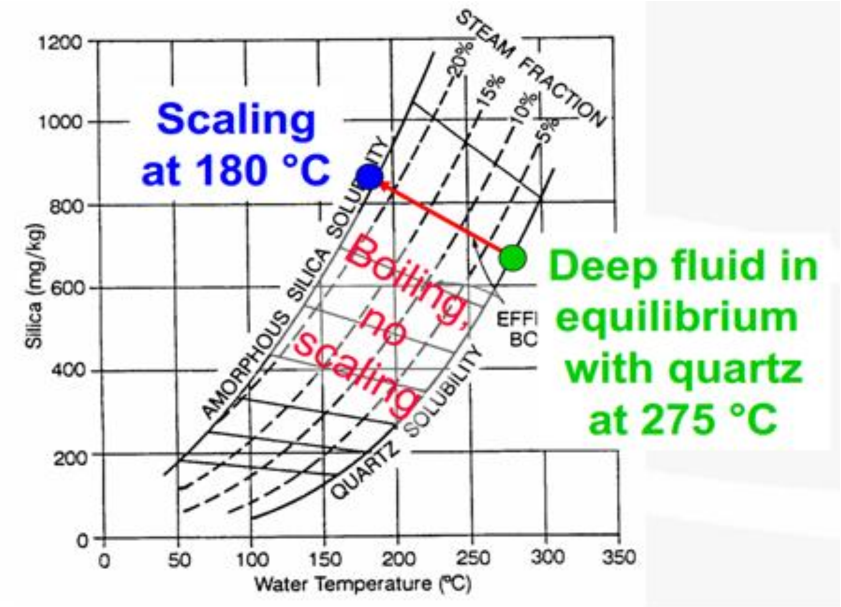


Figure 1.2 : Silica Solubility and Scaling

Lieu (1985) stated that silica scales such as SiO_3 and these generally occur as very finely crystallized deposits of chalcedony or as amorphous opal. They are associated with alkaline or steam flood projects and stem from the dissolution of siliceous formation minerals by high-pH fluids or high- temperature steam condensates. This dissolution can cause poorly consolidated sandstones to collapse or silica to re-precipitate at a distance from the wellbore where the alkalinity, temperature or both of the floods has decreased.

According to Nawrocki and Jacek (1997), silicic acid is a chemical compounds with the chemical formula SiH_4O_4. It is a silanol (a functional group in silicon chemistry with the connectivity Si–O–H. It is related to the hydroxyl functional group (C–O–H) found in all alcohols . Silicic acid is also the general name for a family of chemical compounds containing the element silicon attached to oxide and hydroxyl groups. This family of compound have the general formula $[SiO_x(OH)_{4-2x}]_n$. Some simple silicic acids have been identified, but only in very dilute aqueous solution, such as metasilicic acid(H_2SiO_3), orthosilicic acid (H_4SiO_4, $pK_{a1}=9.84$, $pK_{a2}=13.2$ at 25 °C), disilicic acid ($H_2Si_2O_5$), and pyrosilicic acid ($H_6Si_2O_7$). However in the solid state these probably condense to form polymeric silicic acids of complex structure. Naturally occurring silicic acid is produced by a non-biological process called hydration involving water, and quartz, which is known to be common on Earth. The reaction producing silicic acid from quartz can be written as ***Quartz + Water → Silicic acid*** which can be represented as $\mathbf{SiO_2 + 2\,H_2O \rightarrow SiH_4O_4}$.

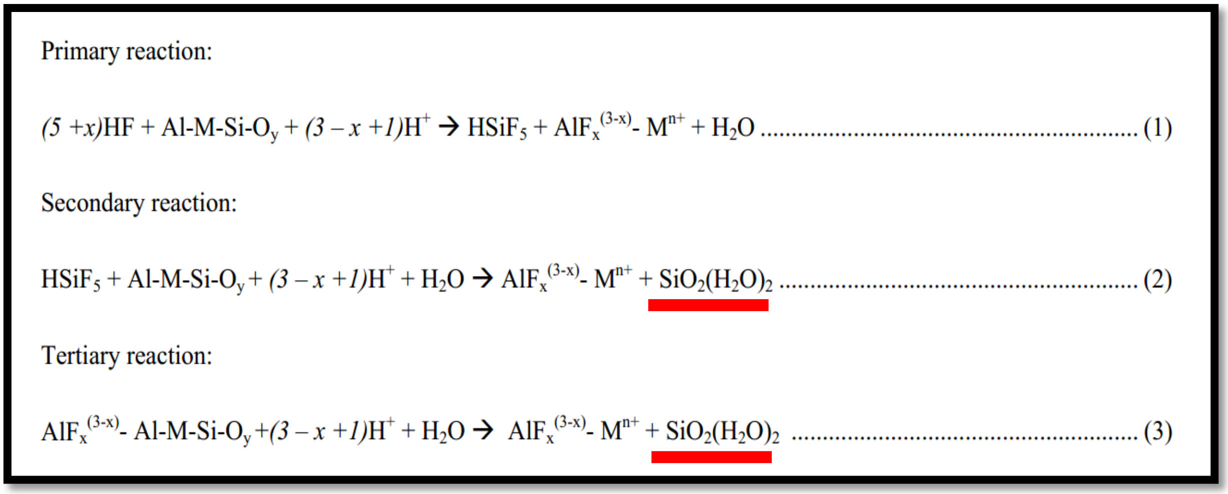
Silicic acid can be produced by the acidification of sodium silicate in aqueous solution. The main problem in using silicic acids in chemical synthesis is that silicic acids readily lose water to form randomly polymeric silica gel, a form of silicon dioxide and such conversion involve condensations (Nawrocki and Jacek, 1997).

The project research focusing to the HF acidizing treatment as most effective acidizing treatments. It employs HF formulations with HCl or organic acids, to dissolve the silicate minerals or scale in the near wellbore region, these studies are directed toward this application. Hydrofluoric acid, HF (used in a blend with HCl – a mud acid system) can be used to dissolve silicate scales (Lieu *et al*, 1985) . Recent studies have shown that the reaction of clay with hydrofluoric (HF) acid is more complex than was earlier believed. It has been found that fluosilicic acid, generated during initial dissolution of clay, reacts with additional clay, extracting aluminum and depositing hydrated silica. It has been suggested that this silica precipitation may result in significant formation damage. This finding are significant **to maximizing resource recovery** because they allow the operator to consider alternatives for treatment processes geared toward higher-temperature wells, fracture acidizing and expansion of the pH range of treatment fluid .Identified decades ago by laboratory studies, the precipitation of

hydrated silica $SiO_2(H_2O)_2$ has been a problem in sandstone acidizing and continues to be a recurring theme in the field of technology (Crowe, 1986).

As mentioned by Thomas *et al* (2002), HF acid is usually used in sandstone-matrix acidizing because of its ability to dissolve a variety of siliceous minerals, including quartz, clay, and feldspar. Sandstone matrix acidizing has been applied in oil, gas and injection wells for decades to remove formation damage in the critical area surrounding the wellbore. The chemical reactions that occur between HF acid systems and the aluminosilicate minerals in sandstone reservoirs have been investigated in detail. It is obvious that the sandstone matrix process is significantly more complex than carbonate acidizing. Unlike carbonate acidizing where the formation damage is simply bypassed via the creation of wormholes, during sandstone acidizing the damage that is plugging the pores normally must be dissolved. Subsequent reactions must not occur that create formation damage i.e. precipitation of calcium fluoride (CaF_2) or potassium fluosilicate. Formation of insoluble reaction products during carbonate acidizing using hydrochloric acid (HCl) is rare, but it occurs in every sandstone acid treatment incorporating an HF acid system i.e. hydrated silica.

Thomas *et al* (2002) mentioned that silica precipitation during a sandstone acidizing treatment or its subsequent deposition post-treatment can negate the effectiveness of said acid treatment. According to Reyes-Garcia (2011), laboratory investigation focused on preventing the formations of silica have been identified a new class of silica polymerization inhibitor (SPI). The potential complication normally incurred during an HF acidizing treatment are precipitation of insoluble byproduct. These byproduct are fluorosilicate and fluoroaluminate which are then possibly react with the Al^+ or Mg^{2+} which are produced during the HF acidizing on sandstone. The analysis indicated that silica polymerization, caused by silicic acid hydrolysis, condensation and polymerization cannot be avoided and the most direct route to minimize it is to maintain an extremely low pH (>2M HCl). For instance, concentration of soluble silica in the range of 100 to 200 mg/L up to 3000 mg/L can be obtained from an acidizing treatment on sandstone materials (Thomas *et al*, 2002) . The concentration obviously is largely dependent of the nature and strength of the acid used to dissolve the aluminosilicate.



Primary reaction:

$(5+x)HF + Al\text{-}M\text{-}Si\text{-}O_y + (3-x+1)H^+ \rightarrow HSiF_5 + AlF_x^{(3-x)} - M^{n+} + H_2O$ .... (1)

Secondary reaction:

$HSiF_5 + Al\text{-}M\text{-}Si\text{-}O_y + (3-x+1)H^+ + H_2O \rightarrow AlF_x^{(3-x)} - M^{n+} + \underline{SiO_2(H_2O)_2}$ .... (2)

Tertiary reaction:

$AlF_x^{(3-x)} - Al\text{-}M\text{-}Si\text{-}O_y + (3-x+1)H^+ + H_2O \rightarrow AlF_x^{(3-x)} - M^{n+} + \underline{SiO_2(H_2O)_2}$ .... (3)

Figure 2.2 :Three consecutive reaction HF acidizing treatment on sandstone

Reyes-Garcia (2011) explain that potential formation of insoluble products arising from ions extracted or leached out of the aluminosilicate during the primary (such as Fe^{2+}, Mg^{2+}) (Gdanski 1997a) and tertiary (Gdanski 1999) reaction AlF_x^{3-x} of HF on aluminosilicates (primary and tertiary) can be controlled by maintaining appropriate HF/HCl concentration and use of chelating agents. The formation of fluorosilicate, proven to be pentafluorosilicate SiF_5^- , is a product reaction of HF and the oxide surface of an aluminosilicate (most likely a clay), this is the secondary reaction in HF acidizing (Gdanski 1997b). In principle, it also can lead to amorphous or colloidal silica precipitation if the pH of the spent fluid was allowed to rapidly increase. HF acidizing treatments, the release of silicon as well as aluminum set off a series of reactions that include the hydrolysis of silicic acid (monomers and oligomers), its condensation and finally polymerization , to form colloidal silica or amorphous silica.

2.2 Problem Arising due to Silicate Scale

Damage due to scale (organic and/or inorganic) deposition is normally characterized by some production patterns:

- Sharp production decline
- Increase in gas/oil ratio (GOR) or water/oil ratio(WOR)
- Reservoir pressure decline relative to bubble point pressure
- Decrease in the API gravity
- Organic deposits on rods and pump

Such problem that can be seen at such as production tubing and surface facilities. Deposition of scale on the tubing can create severe problems to the production system such the common effects:

- Constrained production by reduction of the internal diameter of the tubing. It very often to observe different type of scale in the deposit found on the internal surface of the tubing
- Safety due to the blockage of completion component gas lifts mandrel, SSSV.
- Loss of mechanical integrity due to the presence of corrosion under scale deposit. It is very common to find pitting corrosion and MIC (microbiological induced corrosion) under inorganic scale deposits

Moreover, the presence of inorganic scale complicates the process of calculating, modifying and expanding surface facilities. Thus by accounting for all these variables, we can take a good deal of effort and resources. These issues become a major challenge offshore, especially in deep water. As in tubing, scale can reduce the internal diameter of the flowlines, cause loss of mechanical integrity in subsea and surface facilities, and compromise safety. When scale forms on the bottom of the separators, it reduces the effective volume for separation and interrupt separation of the phases as initially designed .These are the major impact due to deposition in surface facilities:

- Millions of dollars in chemical to prevent, remediate inorganic scale deposition
- Production deferred due to ID reduction
- Poor quality of fluid separation, reduction of the effectiveness of main separators
- reduction in process system (residence time)
- Rotating equipment (pump and compressor) damaged by scales particles
- deposition
- Pipeline leakage for corrosion under deposit of scale

Besides, liquid phase of the fluid and geothermal gases (CO_2, H_2S) must be pumped backwards into the rocks through the re-injection wells in order to maintain pressure in reservoir and decrease influence on the environment. Motion of brine in the heat equipment and reinjection wells is often complicated by silica scaling from solution that decreases amount of obtained energy and leads to the additional expenses for solid material removal. The process of solids formation is of special importance for oil and gas production and transportation systems and needs to be studied and controlled (Kashpura and Potapov, 2000) .

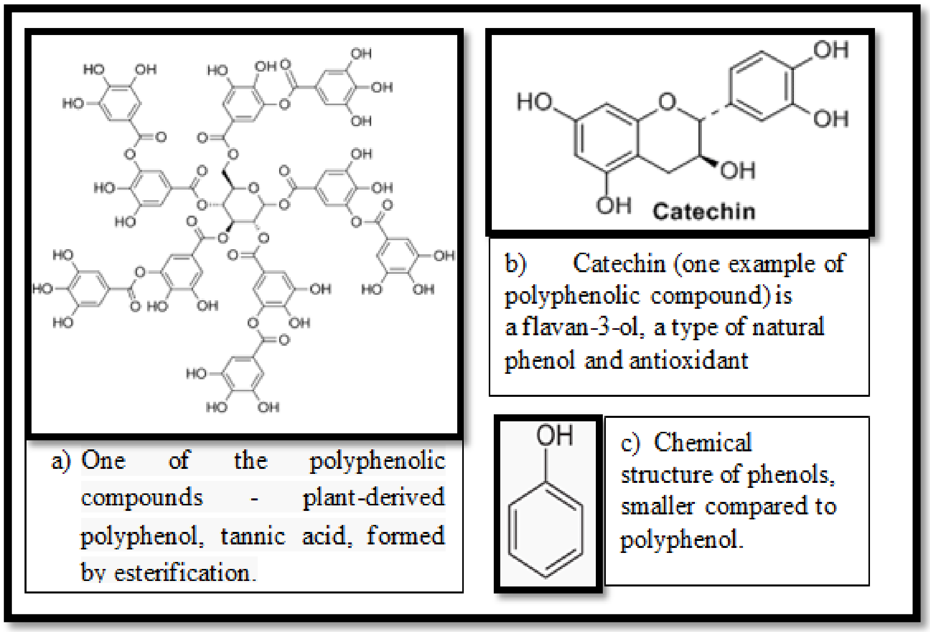
Figure 2.3 : Effect of Silica Rich Scale in Pipeline
Note: Silica rich scales in a pipeline separator station at Reykjanes, Iceland

2.3 Polyphenolic Compounds as Silicate Scale Inhibitors.

Polyphenols which also known as *Polyhydroxyphenols* are a structural class of mainly natural, but also synthetic or semisynthetic, organic chemicals characterized by the presence of large multiples of phenol structural units. The number and characteristics of these phenol structures underlie the unique physical, chemical, and biological (metabolic, toxic, therapeutic, etc.) properties of particular members of the class. They may be broadly classified as phenolic acids, flavonoids, stilbenes, and lignans. According to Maria Teresa (2003), most polyphenols are extracted from natural sources, such as tannic acid from Quercus spp. and the glycoside rutin from Fagopyrum esculentum .

As opposed to smaller phenols, polyphenols are often larger molecules (macromolecules) deposited in cell vacuoles. Polyphenolic compounds consist of phenol (also known as carbolic acid) which is an aromatic organic compounds with the molecular formula C_6H_5OH (Maria, 2003). The molecule consists of a phenyl group ($-C_6H_5$) bonded to a hydroxyl group (-OH). Phenyl groups are closely related to benzene. Phenyl groups have six carbon atoms bonded together in a hexagonal planar ring, five of which are bonded to individual hydrogen atoms, with the remaining carbon bonded to a substituent. Phenyl groups are commonplace in organic chemistry. Although often depicted with alternating double and single bonds, phenyl groups are chemically aromatic and show nearly equal bond lengths between carbon atoms in the ring. Most polyphenols contain repeating phenolic moieties of pyrocatechol, resorcinol, pyrogallol or phloroglucinol connected by esters (hydrolyzable tannins) or more stable C-C bonds (non- hydrolyzable condensed tannins). Proanthocyanidins are mostly polymeric units of catechin and epicatechin (Maria, 2003).

According to Maria (2003), the extraction of polyphenols can be performed using a solvent like methanol, methanol or formic acid, acetic or formic acid. Liquid - liquid extraction can be also performed or countercurrent chromatography. Solid phase extraction can also be made on C18 sorbent cartridges. Besides, other techniques which can be employed are ultrasonic extraction, heat reflux extraction, microwave-assisted extraction, critical carbon dioxide, pressurized liquid extraction or use of ethanol in an immersion extractor. She added that the extraction conditions (temperature, extraction time, ratio of solvent to raw material, solvent and concentrations) have to be optimized. But in this research study, only Soxhlet Extraction can be performed as that is the most method used for isolating polyphenols from solid matrixes.



a) One of the polyphenolic compounds - plant-derived polyphenol, tannic acid, formed by esterification.

b) Catechin (one example of polyphenolic compound) is a flavan-3-ol, a type of natural phenol and antioxidant

c) Chemical structure of phenols, smaller compared to polyphenol.

Figure 2.4 : Chemical structures of polyphenolic compoundss

$$Si(OH)_4^{\circ} + 3H_2L^{\circ} = SiL_3^{2-} + 2H^+ + 4H_2O \quad (K_1) \qquad \text{Equation (1)}$$

The equation above show H_2L which is catechol (polyphenolic compounds) has formed Si-catechol complexes in the solution after reacts with silicic acid $Si(OH)_4$.

Reyes-Garcia (2011) stated that the research is the work details identification of a class of materials for use in acidizing applications in sandstone formations. The use of polyphenolic compounds for silica-polymerization inhibition has been mention in some publication but no information about its range of activity and the amount of silicon it can hold in solution have been reported. Thus, Application and Laboratory Studies of a New Class of Silica Polymerization of Bio-renewable Origin by him from Halliburton has proved the effect of polyphenolic compounds in mitigating silicate scale, which focusing the prevent of earlier stage which is mitigate silica-polymerization.

The material is a naturally occurring substance belonging to the class of tannins (one type of polyohenolic compounds) which obtain from fully renewable sources and biodegradable. As been confirmed by several independent studies that specifically address such characteristic but will not be further discussed here. The polyphenolic compounds displays the capability to sustain in solution high dissolved silicon loading from 0 to 4 pH and at lower concentration silicon concentration up to 5 pH . Related compounds namely dihydroxybenzene and trihydroxy benzene can maintain in solution dissolve silica, though most of these studies have been conducted at a pH of 7 and greater. The polyphenols was prepared from commercially available grade reagent. The stok solution consisted of 20gm/L the pH solution was adjusted to 0.8 with 35% of HCl.

Main objective of the research by Reyes-Garcia (2011) is to show that silicon concentrations in excess of 500 mg/L can be sustained , without precipitation at a pH > 1 .The test conducted were directed towards emulating the sequential introduction of silicon into a solution whose pH is increasing , as in matrix flow during an acidizing treatment. As the pH increases, the free Si-OH group begins to condense and polymerize, eventually forming collodial silica (<0.1 um) and depend on concentration, amorphous silica.

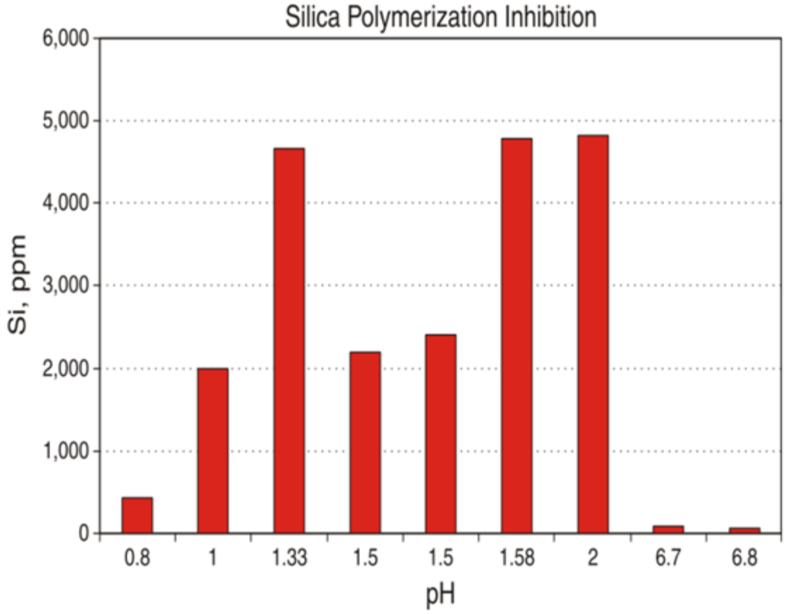


Figure 2.5 : Dissolved silicon concentration as a function pH

Reyes-Garcia (2011) has shown studies of the polyphenolic compounds as functioning to inhibit the condensation and polymerization of silicic acid and higher oligomers into hydrated silica. The higher the concentration of polyphenols the more silicon can be solubilized as the pH increases up to 4.

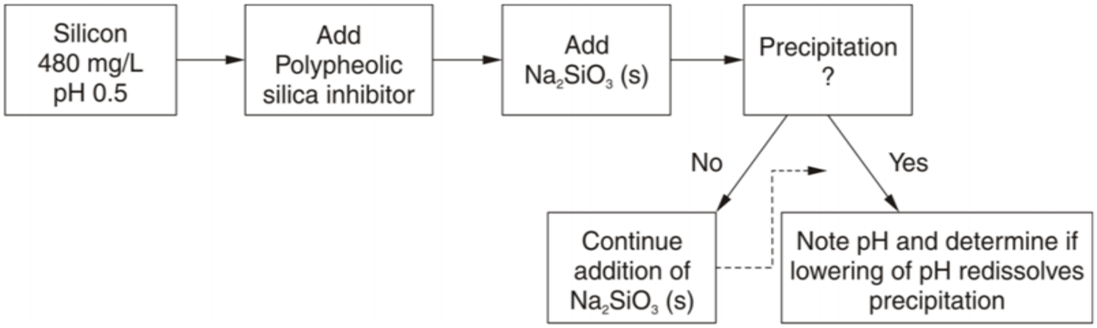


Figure 2.6 : Flow diagram showing path to determine the activity of polyphenolic compounds in minimize or inhibit silicate scale.

In the research also has shown silica, whether collodial or amorphous, can be maintained in solution of concentrations in 2500 mg/L and up to 3500 mg/L when a polyphenolic compounds, in near stoichiometric concentration, is used as a polymerization inhibitor. The polyphenolic compounds is stable in solutions of 0 pH to 5 pH, and effective concentration of 1000 mg/L of the polyphenolic compounds have proven effective in controlling silica precipitation in concentration of <2000 mg/L Si up to 4 pH.

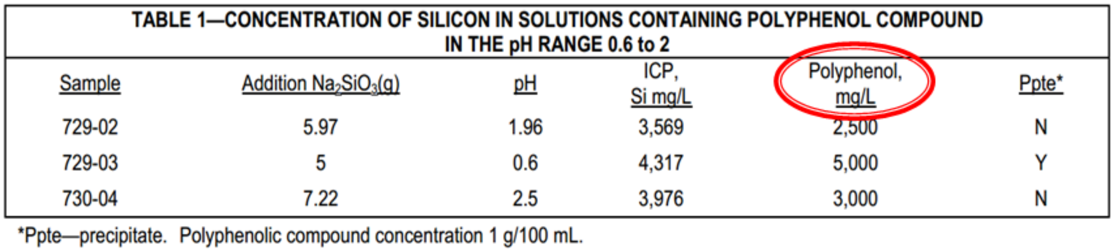


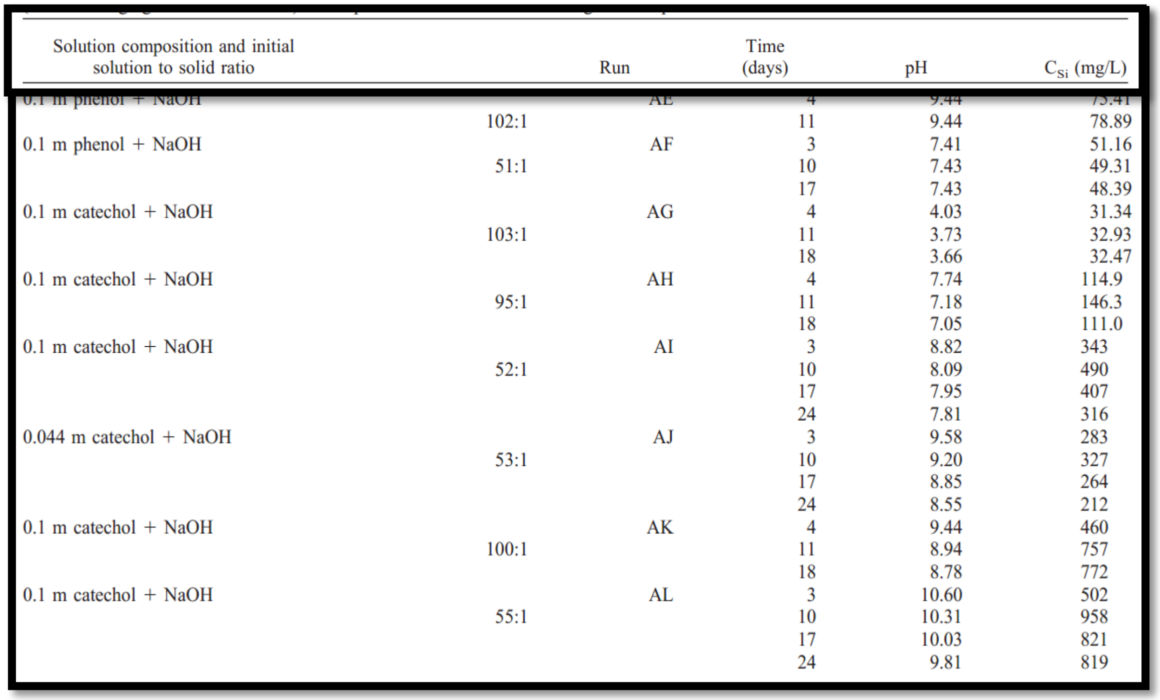
TABLE 1—CONCENTRATION OF SILICON IN SOLUTIONS CONTAINING POLYPHENOL COMPOUND IN THE pH RANGE 0.6 to 2

Sample	Addition Na_2SiO_3(g)	pH	ICP, Si mg/L	Polyphenol, mg/L	Ppte*
729-02	5.97	1.96	3,569	2,500	N
729-03	5	0.6	4,317	5,000	Y
730-04	7.22	2.5	3,976	3,000	N

*Ppte—precipitate. Polyphenolic compound concentration 1 g/100 mL.

Table 2.1: Effect of polyphenols concentration

Cathecol which also grouped in polyphenolic compounds also show the significance in functioning as silica polymerization inhibitor in research study by Pokrovski and Schoot (1998). According to them, the presence of 0.1 m of catechol at pH between 8 and 10, silica solubility increases by an order of magnitude. This increase is accompanied by a significant solution pH change. In most runs, solubility attains a maximum after 1 to 2 weeks and then decrease slightly with time. This decrease is also accompanied by a pH decrease which is likely to be caused by the oxidation of catechol. This shows that it can be apply at higher pH than 7.



Solution composition and initial solution to solid ratio		Run	Time (days)	pH	C_{Si} (mg/L)
0.1 m phenol + NaOH		AE	4	9.44	75.41
	102:1		11	9.44	78.89
0.1 m phenol + NaOH		AF	3	7.41	51.16
	51:1		10	7.43	49.31
			17	7.43	48.39
0.1 m catechol + NaOH		AG	4	4.03	31.34
	103:1		11	3.73	32.93
			18	3.66	32.47
0.1 m catechol + NaOH		AH	4	7.74	114.9
	95:1		11	7.18	146.3
			18	7.05	111.0
0.1 m catechol + NaOH		AI	3	8.82	343
	52:1		10	8.09	490
			17	7.95	407
			24	7.81	316
0.044 m catechol + NaOH		AJ	3	9.58	283
	53:1		10	9.20	327
			17	8.85	264
			24	8.55	212
0.1 m catechol + NaOH		AK	4	9.44	460
	100:1		11	8.94	757
			18	8.78	772
0.1 m catechol + NaOH		AL	3	10.60	502
	55:1		10	10.31	958
			17	10.03	821
			24	9.81	819

Table 2.2 : Final concentration of silicon in solution containing catechol and phenol

From Pokrovski and Schoot (1998), both phenols and cathecol shows good result in maintaining the silicic acid from being condensed and polymerized in pH higher than 7 (pH 8-11 range) which might indicated the situation of other fluid flooding after acidizing treatment in hydrocarbon well employed.

The SPI mechanism is disruption or retardation of the condensation and polymerization of silicic acid monomers and oligomers and the ultimate growth of amorphous silica. Thus polyphenolic compounds are becoming promising substance according to the research for maintaining the free silicic acid from condensing and polymerize into hydrated silica, eventually help to prevent silicate scale during operation of acidizing treatment.

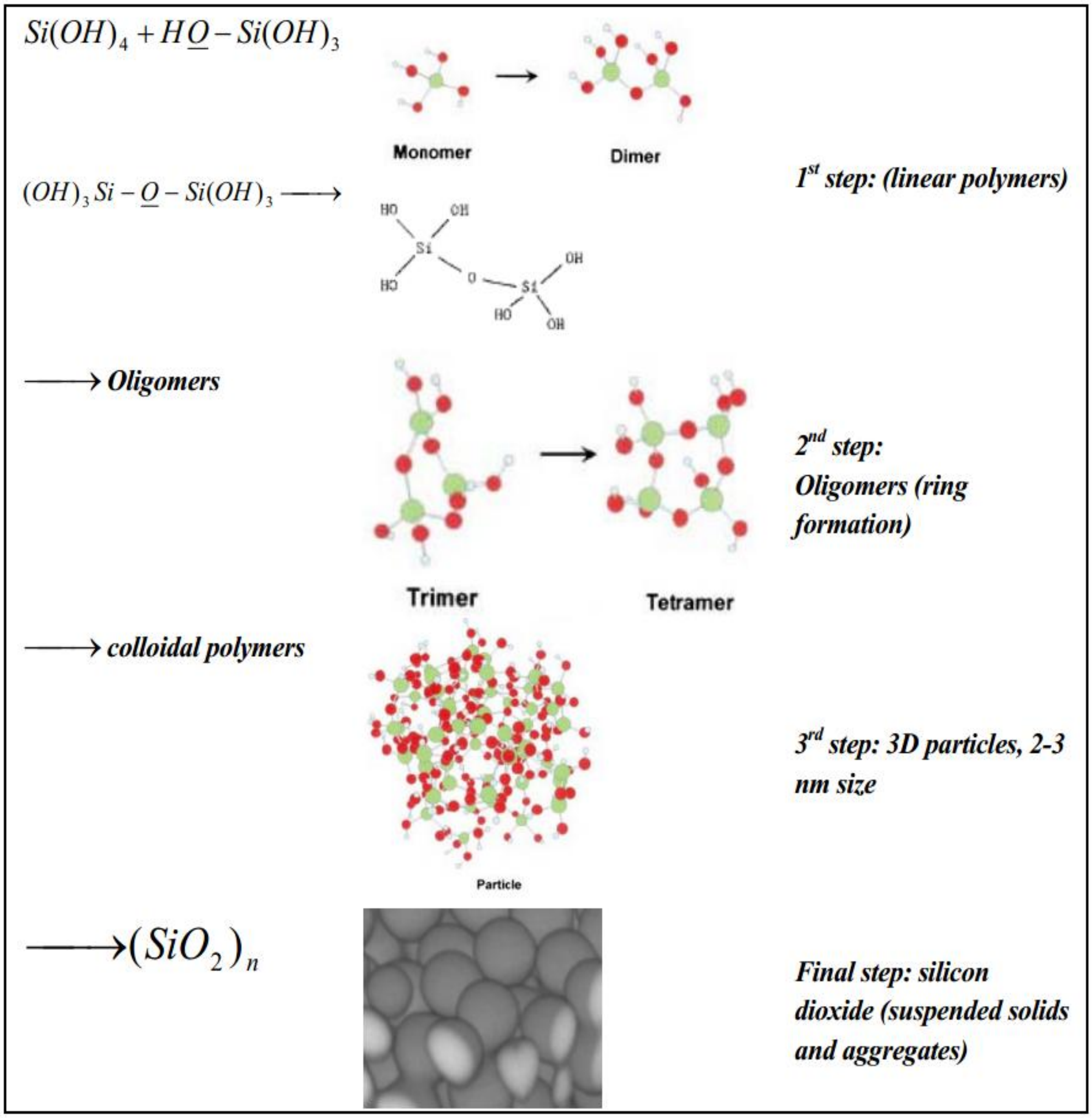


Figure 2.7 : Stepwise formation of colloidal silica particles from soluble silicic acid

2.4 Plants Identified With Polyphenolic Compounds.

The identified plants are :

- Camellia Sinensis (Green tea)
- Piper Betle (betel)
- Premna Cordifolia (Bebuas)
- Abelmoschus Esculentus (Okra)

2.4.1 Premna Cordifolia (Bebuas)

Figure 2.8 : Premna Cordifolia (Bebuas)

Bebuas tree has its own benefits to health. A decoction of herbs and sprouts Bebuas trusted by the Malays to cure a headache problem. Raw sprouts blanched or boiled and eaten with sambal belacan, budu and cencaluk. It tastes sweet and little spicy and crunchy and bitter overtones. The herb refers to the soft-stem shrubs. Normally herbal plants have soft stems and succulent. It can be eaten to increase appetite, milk production, body treatment windy and dysfunction. Children who have trouble with parasitic worms (helminths) in the body can be fed to treat illness. According to Shukri *et al* (2011), bebuas were screened for their phenolic compounds and antioxidant activities.

2.4.2 Abelmoschus Esculentus (Okra)

Figure 2.9 : Abelmoschus Esculentus (Okra)

Okra or lady finger is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. Okra is good for brain development which strengthens memory and brain fitness. In traditional medicine, seeds, roots and flowers and drink boiled okra, able to urination, reduce swelling or inflammation, reduce hard labor, and cure gornea and syphilis. Boiling water is also suitable to be applied to various kinds of sores and boils. Mixed broth can be made sugar syrup and sore throat treatments. Mature okra seeds also have the potential for the process to obtain high-quality vegetable oils and fried can also be processed to be used as a coffee drink. Unspecified parts of the plant were reported in 1898 to possess diuretic properties. Polyphenols, a heterogeneous group of compounds, are one of these constituents. From the PubMed article which describes the polyphenol composition and antioxidant potential of H. esculentus of Nigerian origin, Quercetin glucoside (quercetrin) and an unidentified flavonoid were detected.

2.4.3 Camellia Sinensis

Figure 2.10 : Camellia Sinensis (green tea)

Camellia Sinensis is native to East, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions. It is an evergreen shrub or small tree that is usually trimmed to below 2 m (6.6 ft) when cultivated for its leaves. The leaves have been used in traditional Chinese medicine and other medical systems to treat asthma (functioning as a bronchodilator), angina pectoris, peripheral vascular disease, and coronary artery disease. Recent medical research on tea (most of which has been on green tea) has revealed various health benefits, including anti-cancer potential, effects on cholesterol levels, antibacterial properties and positive effects for weight loss. It is considered to have many positive health benefits due to tea's high levels of catechins, a type of antioxidant. Green tea is prepared in such a way as to preclude the oxidation of green leaves polyphenols. Fresh tea leaves are unusually rich in the flavanol group of polyphenols known as **catechins** which may constitute up to 30% of the dry leaves weight. PubMed stated that other polyphenols include flavanols and their glycosides, and depsides such as chlorogenic acid, coumarylquinic acid, and one unique to tea, theogallin (3-galloylquinic acid).

2.4.4 Piper Betle (Sirih)

Figure 2.11 : Piper Betle (Sirih)

The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves and white catkin. Betel requires high land and especially fertile soil. Waterlogged, saline and alkali soils are unsuitable for its cultivation. Betel leaves are often used in a variety of Malay customs. In medical field, betel help to reduce sinus and daylight vision, face refreshing and cleansing, remove underarms, relieves itching and red eyes due to irritation and stop bleeding gums .Extraction process was conducted on locally collected Piper betle leaves using petroleum ether and methanol. Essential oil was also extracted using hydrodistillation and analysed by GC-MS. Two major components isolated from the petroleum ether extract were identified as chavibetol [2-methoxy-5-(2-propenyl)phenol] and β-sitosterol while one major component isolated from methanol extract was identified as allylpyrocatechol [4-(2-propenyl)- 1,2-benzenediol]. Polyphenols, flavonoids and the reducing power of treated and untreated.

2.4.5 Conventional Inhibitor (PASP)

Figure 2.123 : PASP Chemical

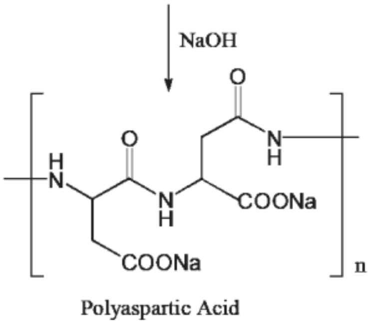


Figure 2.134 : PASP Chemical Structure

As the study to investigate the effectiveness of green inhibitor extracted from the four samples, thus a benchmark should be employed. Norbert *et al* (2004) stated that PASP (polyaspartate) has been chosen as poly (amino-acids) and in particular polyaspartates have already been proposed as environmental safe "green" mineral scale inhibitors and also field tested in squeeze operations. Besides, Robert *et al* (1996) explains that the impact of chemicals on the environment is an issue of increasing global importance, thus biodegradability, the capability of being broken down into simple, non-toxic materials by the action of microorganisms and fungi, is an important mechanism for limiting the build-up of chemicals in the environment.

Thus, PASP which are highly biodegradable alternatives to polyacrylate based scale inhibitors in other research study, as a benchmark for the green inhibitor in the four samples in this project research. It can be employed as it is also widely used in oilfield operation and due to its water-solubility and ability to chelate metal ions, polyaspartate is used as a biodegradeable anti-scaling agent and a corrosion inhibitor. Polyaspartates act as complexes with the metal ions which delay scales which have same mechanism as the polyphenolic compounds . The PASP is obtained from Shandong Taihe Water Treatment Co. Ltd. According to David *et al* (2011), PASP is commercially available and has numerous applications. The main uses of PASP are as a scale and corrosion inhibitor in water treatment plants and low - temperature cooling towers, as a biodegradable scale control chemical for oil field and other mining operations, as a green chemical for detergent formulations, and as a fertilizer synergistic agent promoting nutrients absorption by crops.

CHAPTER 3 - RESEARCH METHODOLOGY

Chapter 3 elaborates the methodology, key miletones, project gantt chart, samples preparation, compatibility, turbidity and pH test procedure.

3. 1 Methodology Flow Chart

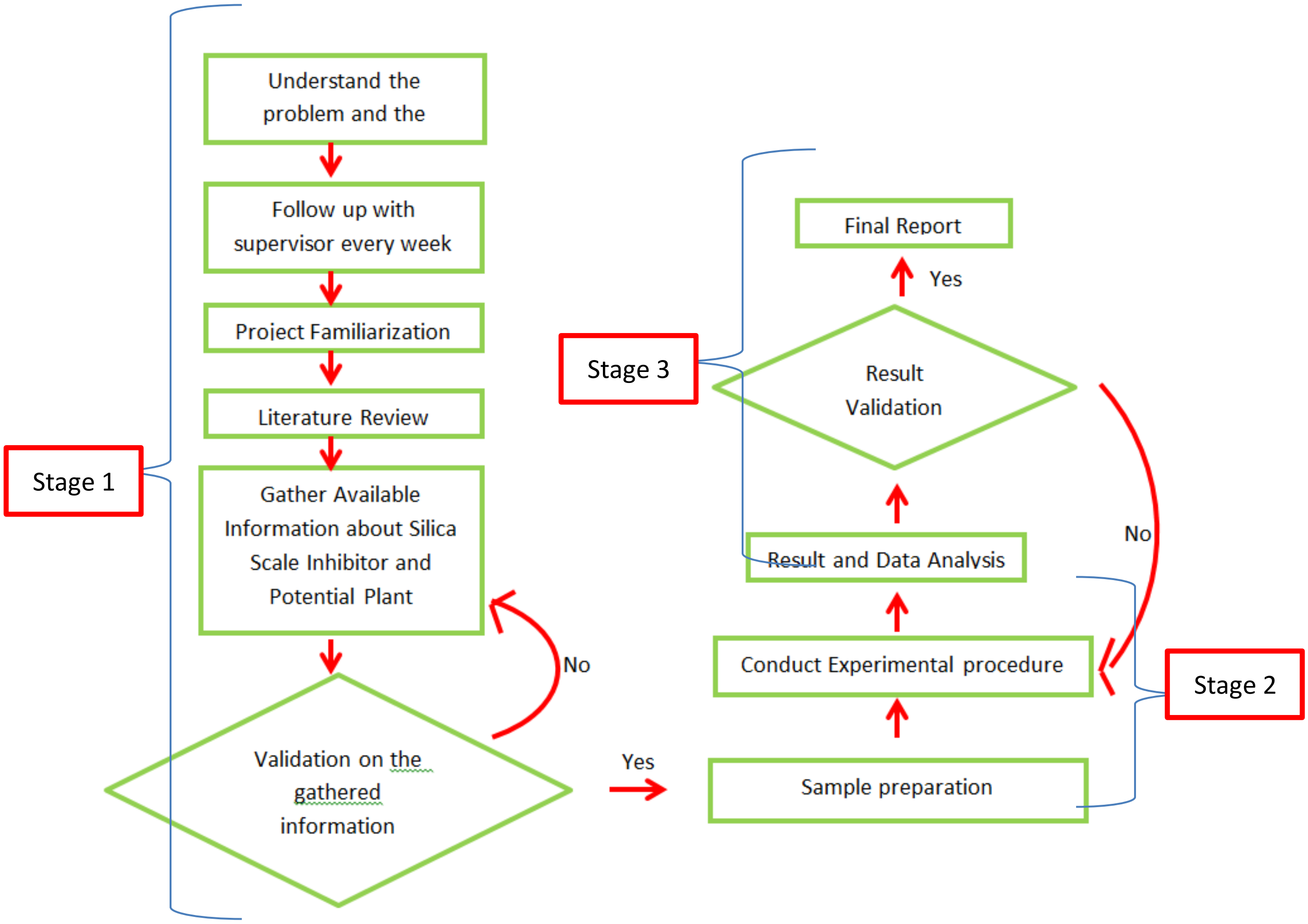
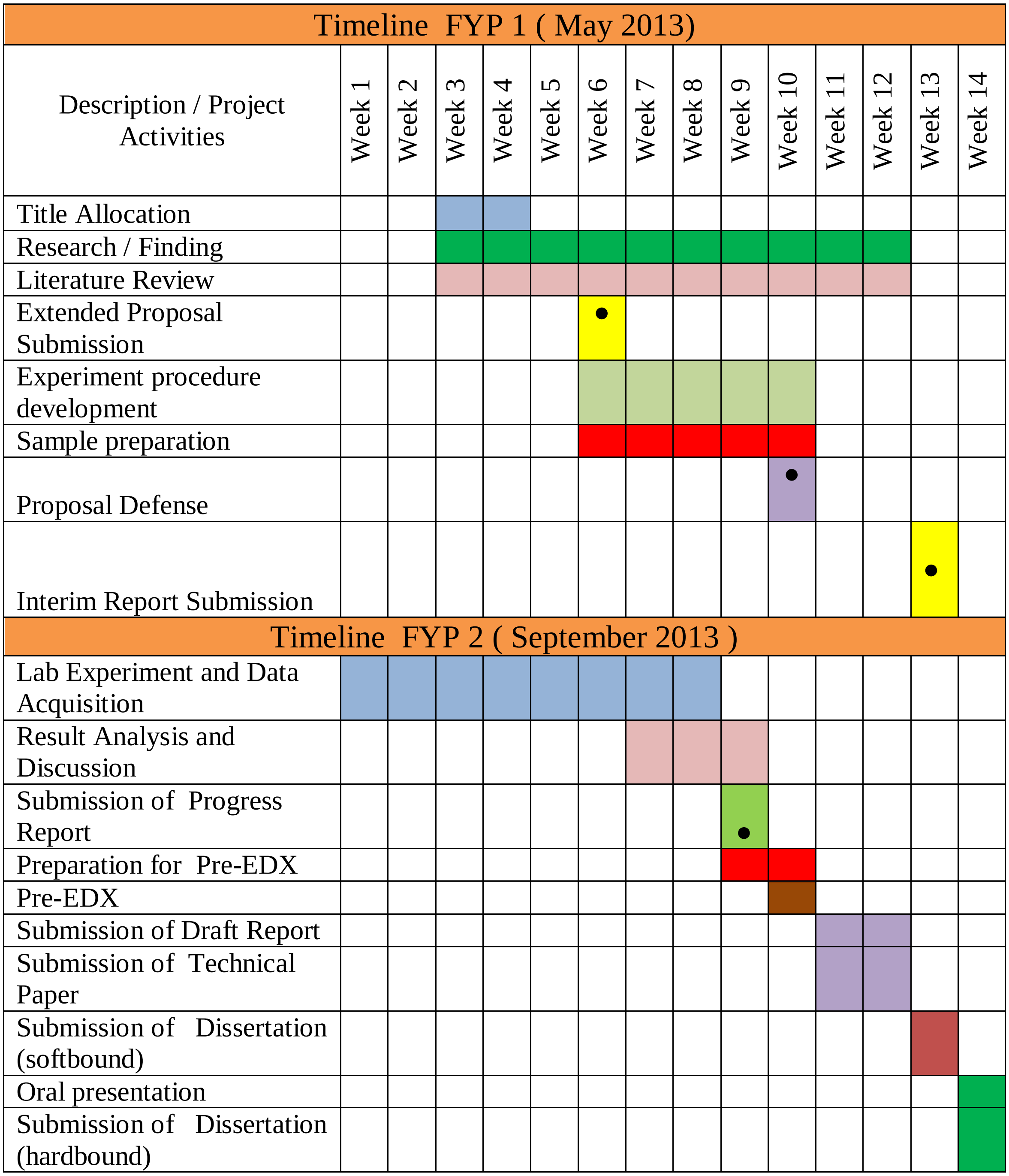


Figure 3.1 : Flow Chart of Research Methodology

Figure 3.1 shows the stages and planned activities for this project in general for the two semesters. Specifically , four experiments had been planned for this project within specifies time spans which taking about three months to be completed in second stages Unfortunately , due to the unavailability of XRF equipment and Silico-molybdate reagent respectively , thus ensuring to proceed with three other experiments which can be done. After data acquisition, result will be analyzed to come up with conclusion, discussion and recommendation which are the outcomes of this research study.

3.2 Project Research Gantt Chart

Table 3.1 : Project Gantt chart



Timeline FYP 1 (May 2013)														
Description / Project Activities	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14
Title Allocation			🟦	🟦										
Research / Finding			🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩		
Literature Review			🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥		
Extended Proposal Submission						🟨 ●								
Experiment procedure development						🟩	🟩	🟩	🟩	🟩				
Sample preparation						🟥	🟥	🟥	🟥	🟥				
Proposal Defense										🟪 ●				
Interim Report Submission													🟨 ●	
Timeline FYP 2 (September 2013)														
Lab Experiment and Data Acquisition	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦						
Result Analysis and Discussion							🟥	🟥	🟥					
Submission of Progress Report									🟩 ●					
Preparation for Pre-EDX									🟥	🟥				
Pre-EDX										🟫				
Submission of Draft Report											🟪	🟪		
Submission of Technical Paper											🟪	🟪		
Submission of Dissertation (softbound)													🟥	
Oral presentation														🟩
Submission of Dissertation (hardbound)														🟩

3.3 Key Milestones

The key milestones are explained in chart which has been incorporated at Appendix 1 and 2.

3.4 Experimentation Overview

3.4.1 Samples Preparation

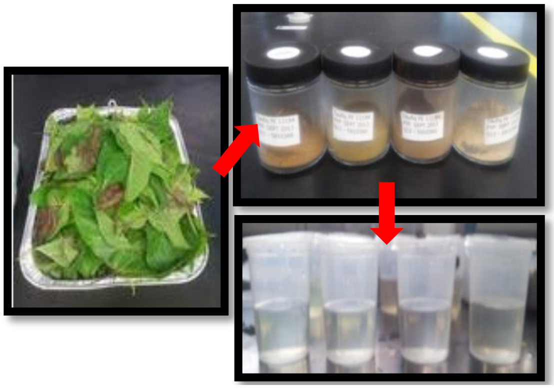


Figure 3.114 : Sequence from the plants to extracted scale inhibitors (polyphenolic compounds)

As being stated before, the following identified green plant samples which have been extracted:

1. *Camellia sinensis* (Teh),
2. *Premna cordifolia* (Bebuas)
3. *Piper Betle* (Sirih)
4. *Abelmoschus esculentus* (Kacang Bendi).

Sample 1 was obtained from plantation at Cameron Highland, Perak while sample 2,3 and 4 were obtained from the house garden in Alor Star, Kedah.

3.4.1.1 Removal Chlorophyll (green colour) from the four samples.

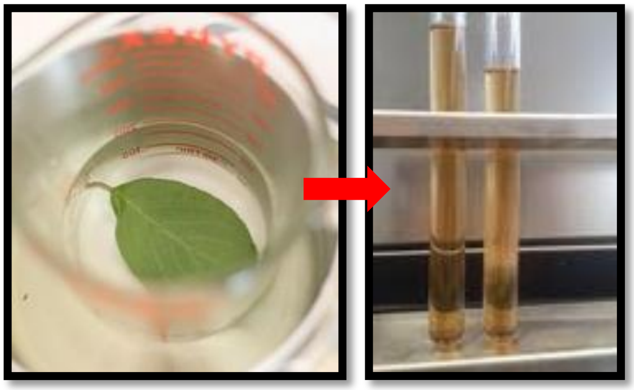


Figure 3.2 : Removal of greenish colour of leaves

1. The plants were thoroughly washed under a running tap water to remove all the dirt.
2. Pour tap water into a quarter of wide-sized pot and set it on the heater. Turn the heat setting to high.
3. Bring the water to a boil and set your leaves in it for two minutes.
4. Remove the pot from the heat. Use a slotted spoon to take the leaves out.
5. Pour 1 cup rubbing ethanol into a tall, heat-safe beaker and set it in the middle of the pot of hot water. Do not spill the water from the pot into the beaker.
6. Place the leaves in the glass with the rubbing ethanol. Make sure the leaves are entirely covered with the rubbing ethanol.
7. Wait one hour and return to check on the leaves. The leaves should now show its true color. The rubbing ethanol will now be approximate green. The green in the rubbing ethanol is the removed chlorophyll.

3.4.1.2 Samples Drying and Grinding

Figure 3.4 : Process of drying up the leaves.

The samples were dried without using sunlight to prevent other reaction towards the sample before being extracted. The samples were dried in the oven overnight.

Figure 3.5 : Process of milling the dried sample into powder form.

The powdered was sieved and stored in plastic bottle at room temperature before proceed with Soxhlet Extraction of polyphenolic compounds.

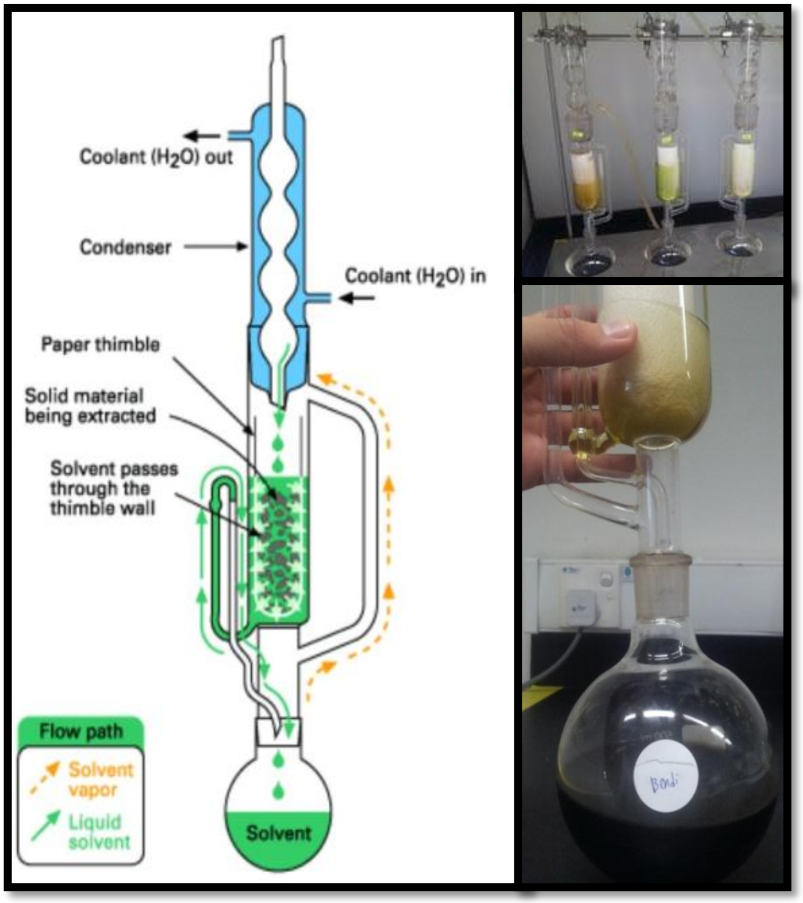


Figure 3.6 : Process of extraction from powdered sample by using Soxhlet Extraction

3.4.1.3 Sample Extraction

Soxhlet Extraction Procedure:

1. The sample is placed in a porous cellulose thimble.
2. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser.
3. The flask is heated and the solvent evaporated and moves up into the condenser where it is converted into a liquid that trickles into extraction chamber containing the samples.
4. The extraction chamber is designed in such way that when the solvent surrounding the sample exceeds a certain level, it overflows and trickles back down into boiling flask.
5. At the end of extraction process, which last for few hours depending on the volume of extract required, the flask containing the solvent and sample is removed.

3.4.1.4 Samples Purification

Rotary evaporator at Academic Blok 4 is used to separate the methanol from the extracted samples. Heating the extracted samples above the boiling point of 148.5°F (64.7°C) allowed only removal of methanol from the mixture in extracted samples. The volume of methanol used during the Soxhlet extractor should be the same after being extracted from the mixture of polyphenolic compounds and methanol.

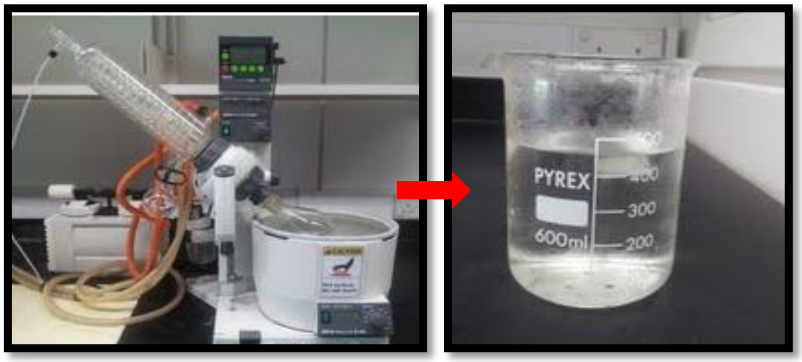


Figure 3.715 :Process of separation methanol from the mixture with polyphenolic compounds using Rotary Evaporator

Table 3.2 : Materials, Tools and Equipment for Samples Preparation

Chemicals	Tools / Apparatus	Equipment
• 200 Methanol for each sample • ml 95% Ethanol	• 1 Litre Beakers • Measuring Cylinders • Spatula , Aluminium foil • Plastic and Metal container • Alundum Extraction Thimble • Wide pot , Funnels , Sieve • Test Tubes and Test Tube Rack	• Laboratory Oven • Soxhlet Extractor • Rotary Evaporator • Electronic Weighing Scale • Granulator • Mortar Grinder

3.4.1.5 Synthetic Water (Brine) Preparation

Table 3.3 : Composition of Synthetic water (brine)

Salts	Concentration gram/Liter	ppm
Sodium Chloride (NaCl)	3.5	3500
Potassium Chloride (KCl)	0.2	200
Magnesium Chloride ($MgCl_2.6H_2O$)	2.5	2500
Calcium Chloride ($CaCl_2.2H_2O$)	0.6	600

3.4.1.6 Silicate Solution Preparation

Table 3.4 : Composition of Silicate solution

Salt	Concentration gram/Liter	ppm
Sodium Metasilicate (Na_2O_3Si)	a) Low = 0.1	100
	b) High = 0.2	200

3.4.2 Compatibility Test

The first step in initialized this experimental conduct , there is need to check whether or not the inhibitor precipitates when mixed with the synthetic water (brine) which will cause damage to the following experiments. This test is assessed by visually examination of the sample initially at room temperature and after 24 hours being kept in the electronic oven at 90 ˚ Celcius in order to simulate the experiment at reservoir temperature.

Table 3.5 : Materials, Tools and Equipment for Compatibility Test

Chemicals	Tools / Apparatus	Equipment
• 250 ml of synthetic water (brine)	• 4 test tube for each samples • 250 ml Measuring Cylinders • Dropper	• Laboratory Oven

3.4.3 Turbidity Test

Turbidity is measured by applying Nephelometry, a technique to measure level of light scattered by the particles at right angles to the incident light beam. The scattered light level is proportional to the particle concentration in the sample. The unit of expression is *Nephelometric Turbidity Unit* (NTU). If a large amount of suspended solids are present in water, it will appear turbid in appearance. The turbidity depends upon fineness and concentration of particles present in water. Thus in this experiment , as the silicic acid combined with metal ion for example magnesium or calcium, the solution will have increase in turbidity. The four samples and one conventional biodegradable inhibitor (PASP) as benchmark is being tested.

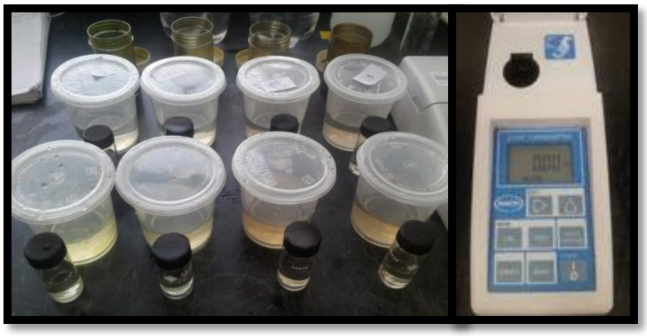


Figure 3.8 : Turbidity test of four sample at different concentration

Table 3.6: Materials, Tools and Equipment for Samples Turbidity Test

Chemicals	Tools /Apparatus	Equipments
• Sodium Chloride • Potassium Chloride • Magnesium Chloride • Calcium Chloride • Sodium Metasilicate • Distilled Water	• 1 Litre Beakers • Measuring Cylinder Spatula • Plastic container • Funnels & Pipette • Test tube rack	• pH meter • Turbidimeter • Electric Weighing Scale • Heater & Magnetic Stirrer

3.4.4 pH Test

Figure 16 : pH meter and buffers.

pH test is conducted as a variable of conditions in the mixtures of the silicate solution and synthetic water in order to measure the performance of those four green inhibitor samples. According to Reyes-Garcia (2011), the polyphenolic compounds is stable in solutions of 0 to 5 pH, and effective concentration of 1000 mg/L of the polyphenolic compounds have proven effective in controlling silica precipitation in concentration of <2000 mg/L Si up to 4 pH. Thus, range from pH 1 to 5 is used in order to simulate matrix flow condition during HF acidizing treatment.

Table 3.7 : Materials, Tools and Equipment for Samples pH Test

Chemicals	Tools / Apparatus	Equipment
• Buffer (pH 4 , 7, 10) • Diluted Sulphuric acid ,H_2SO_4 • Sodium Hydroxide , NaOH	• 4 test tube for each candidate • 250 ml Test tubes	• pH meter • pipette

CHAPTER 4 : RESULT & DISCUSSION

The experimental polyphenolic compounds as silicon polymerization inhibitor was employed using method of elemental analysis which was conducted by coupled plasma-optical emission spectroscopy (ICP-OES). But in this project, mostly the result are determined by the turbitidy value (NTU) which has been effected by the reaction of scale solution and inhibitors. Chapter 4 elaborates about the experimental result obtained and detail discussion .

4.1 Compatibility Test

Table 4.14: Initial and Final Observation of Compatibility in Synthetic water (brine)

Green Silicate Inhibitor	Concentration (ppm)	Initail Observation at room temperture	Final Observation at 90˚ Celcius after 24 hours
Camellia sinensis (Teh)	100	Clear Solution	Clear Solution
	500	Clear Solution	Clear Solution
	1000	Slightly mild greenish clear Solution	Slightly mild greenish clear Solution
	5000	Greenish Clear Solution	Greenish Clear Solution
Premna cordifolia (Bebuas)	100	Clear Solution	Clear Solution
	500	Clear Solution	Clear Solution
	1000	Slightly mild greenish clear Solution	Slightly mild greenish clear Solution
	5000	Greenish Clear Solution	Less Greenish Clear Solution than initially
Piper Betle (Sirih)	100	Clear Solution	Clear Solution
	500	Clear Solution	Clear Solution
	1000	mild greenish clear Solution	Slightly mild greenish clear Solution
	5000	Greenish Clear Solution	Greenish Clear Solution
Abelmoschus esculentus (Kacang Bendi)	100	Clear Solution	Clear Solution
	500	Clear Solution	Clear Solution
	1000	Slightly mild greenish clear Solution	Slightly mild greenish clear Solution
	5000	Greenish Cloudy Solution	Greenish Solution

Note : The visual observation of the compatibility test can be refer at Appendix 15.

As the polyphenolic compounds does not having any precipitation in the synthetic water (brine) thus, the consequent experiment to test the performance of this green inhibitor from four samples would be proceed without any problems. The mild greenish colour is due to the left over chlorophyll which cannot be eliminated 100 percent although the plant has been soaked with 95% ethanol. These consequent somehow impaired the result of clear solution. If the green colour is successfully being eliminated, thus it will yield good result and turbidity test will also does not being affected (low reading can be acquired).

4.2 Turbidity Test

Starting from no inhibitor to high inhibitor concentration of green inhibitor, the graph shows the more the inhibitor employed the lesser the turbidity reading. We can use this table and graph below to analyze the following experimental result in detail at the next continuing pages.

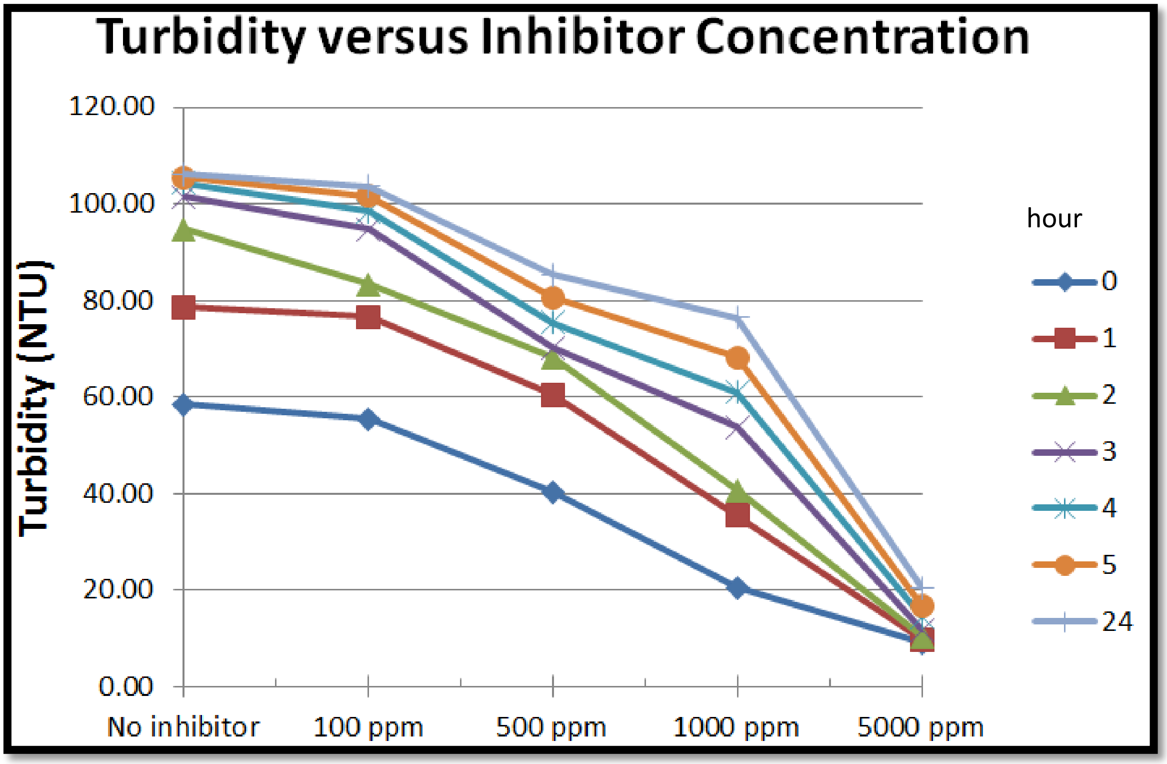


Figure 4.1 : Concentration of the conventional inhibitor (PASP) and turbidity

Note : The tabulated data of the graph above can be referred at Appendix 7.

The scale inhibitors were test in mixture of 50 ml **of 100 ppm silicate solution** and 50 ml of synthetic water (brine).

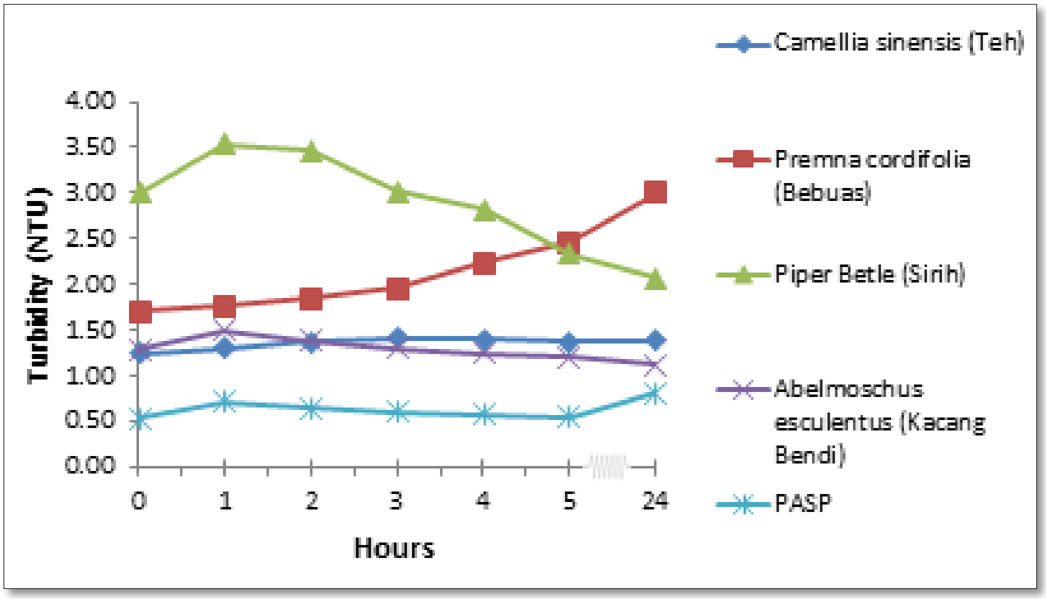


Figure 17 : Turbidity (NTU) of 100 ppm scale inhibitor concentration

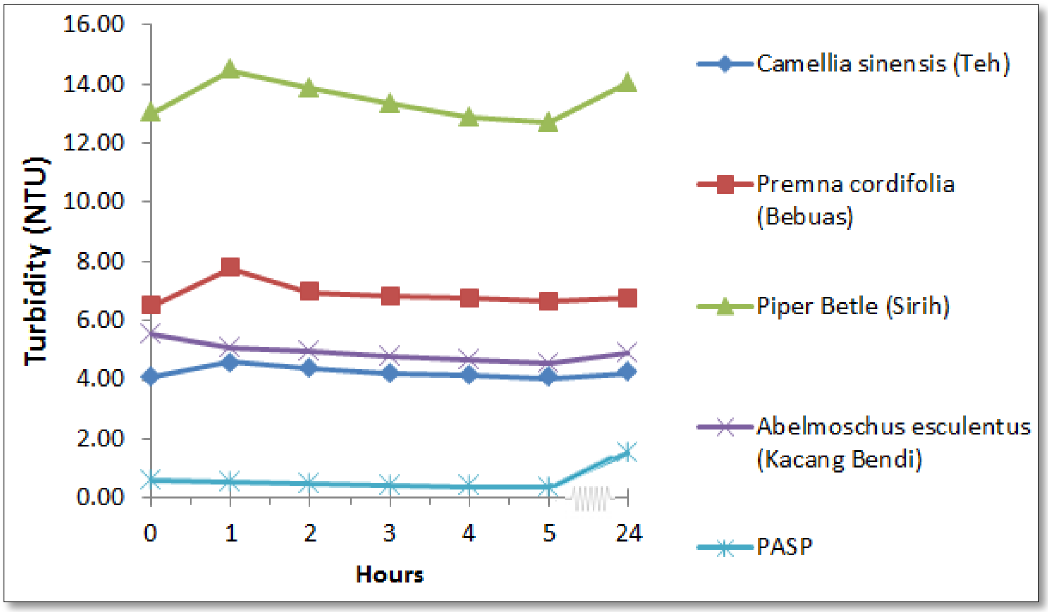


Figure 4.3 : Turbidity (NTU) of 500 ppm scale inhibitor concentration

Note : The tabulated data can be referred at Appendix 9 and 10.

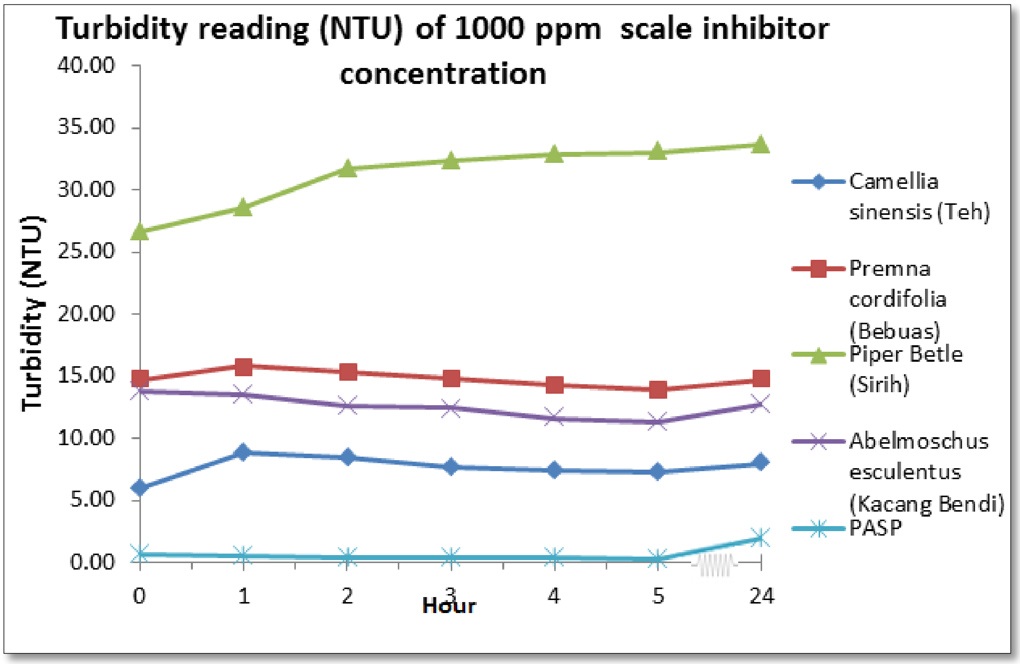


Figure 4.4 : Turbidity (NTU) of 1000 ppm scale inhibitor concentration

Note : The tabulated data can be referred at Appendix 11.

As shown in three consecutive figures and tables above, all the four samples shows higher turbidity than PASP. This is because the green colour which cannot be eliminated 100 percent from the identified plants. As the turbidity measure the "cloudiness" of the solution, the green colour does gives effect to NTU reading. The highest turbidity reading yield from the Piper Betle is 33.67 NTU. The turbidity reading of PASP as the benchmark showing the decreasing value when testing with the three concentration 100 ppm, 500 ppm and 1000 ppm.

The higher turbidity shows that there are high compounds formation in the solution, while low turbidity shows lesser compounds formed from the silicate ion and metal ion. This proves that the PASP directly inhibit the silicates compounds to be formed for the first five hours. After 24 hours, the turbidity value seem higher that initial. This explains that PASP only prevent the reaction of the metals ion to react with the silicate ions by forming complexes with the silicic acid or silicate ions. The increasing value of turbidity showing that there is not enough polyphenolic compounds amount in the extracted plant in order to form complexes with the silicic acid or silicate ions in the mixed solution. Discussion of four different samples will be elaborate at the next page.

For the 100 ppm silicate solution used, the discussion has summarized the four samples performance as follow:

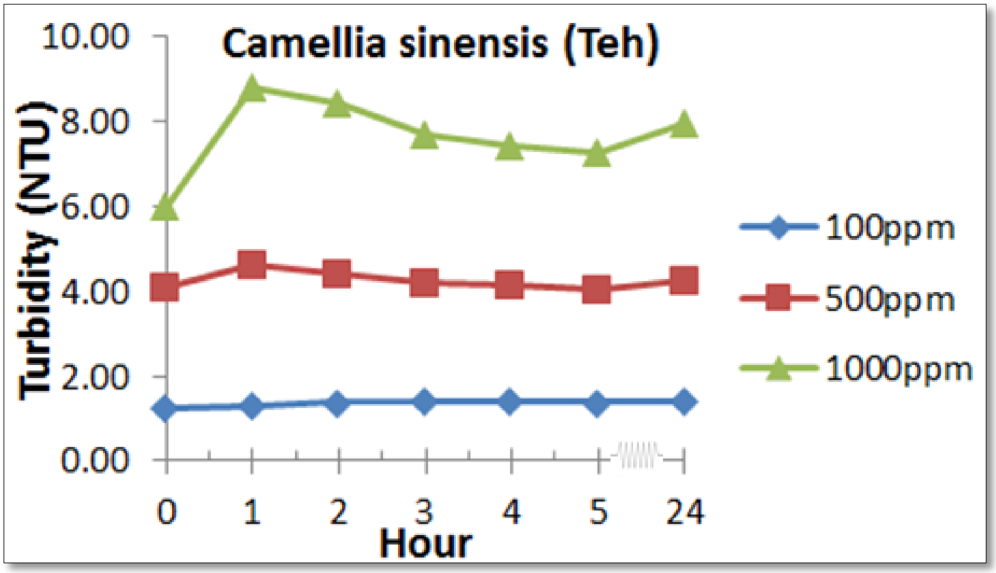


Figure 4.5 : Performance of Camellia sinensis at 100 ppm, 500 ppm and 1000 ppm tested with 100 ppm silicate solution

For Camellia sinensis 100 ppm concentration of extracted solution is not enough to decrease the turbidity value. The insufficient of polyphenolic compounds to form complexes with the silicic acid or silicate ions can be assumed. The turbidity reading shows slow increment form 1.25 NTU till 1.38 NTU after 24 hours.

At 500 ppm concentration of Camellia sinensis, the result shows a good decreasing turbidity value curve. This show that the sufficient polyphenolic compounds extracted from it has good potential to become best plant contain green inhibitor as all the turbidity reading is generally low than the other samples.

As the concentration increases, the slope of decreasing in turbidity value becomes clearer. Although the 1000ppm overall turbidity value is much higher than 500ppm and 100ppm concentration used, the effect is very significant for the first five hours. The overall higher value of turbidity in 1000ppm is due to the high concentration of the green coloured extracted solution which cannot be removed completely during sample preparation.

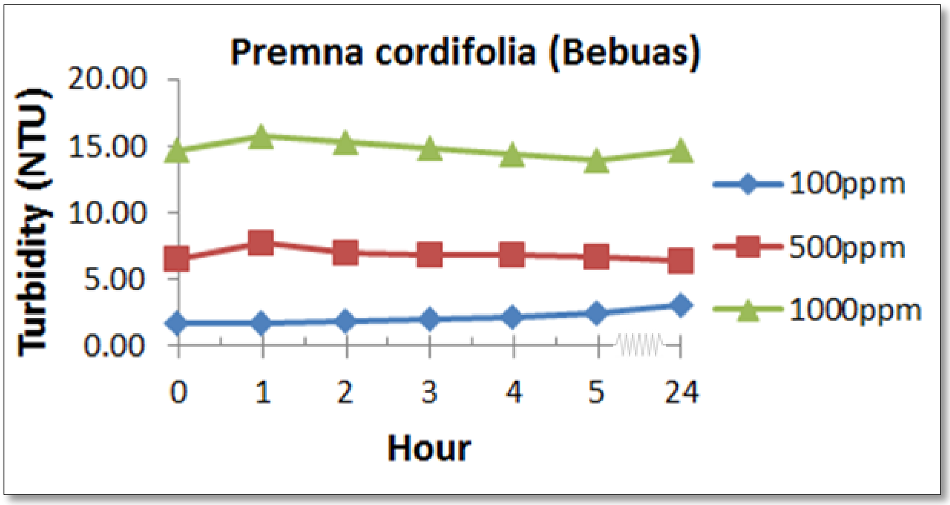


Figure 18 : Performance of Premna cordifolia (Bebuas) at 100 ppm, 500 ppm and 1000 ppm tested with 100 ppm silicate solution

Premna cordifolia (Bebuas) show same characteristic with Camellia sinensis (Teh) as the turbidity value curve slightly similar. The differences between these two samples are the large values of turbidity of Premna cordifolia compared to the Camellia sinensis. From this, we can assume that Premna cordifolia does not have as many polyphenolic compounds amount as Camellia Sinensis contained. Besides the higher concentration of green colour of Premna cordifolia than Camellia sinensis does become contributing factor to the large value of turbidity. For example the maximum turbidity value for Camellia sinensis at 1000 ppm is 8.80 NTU while for Premna cordifolia is 15.80.

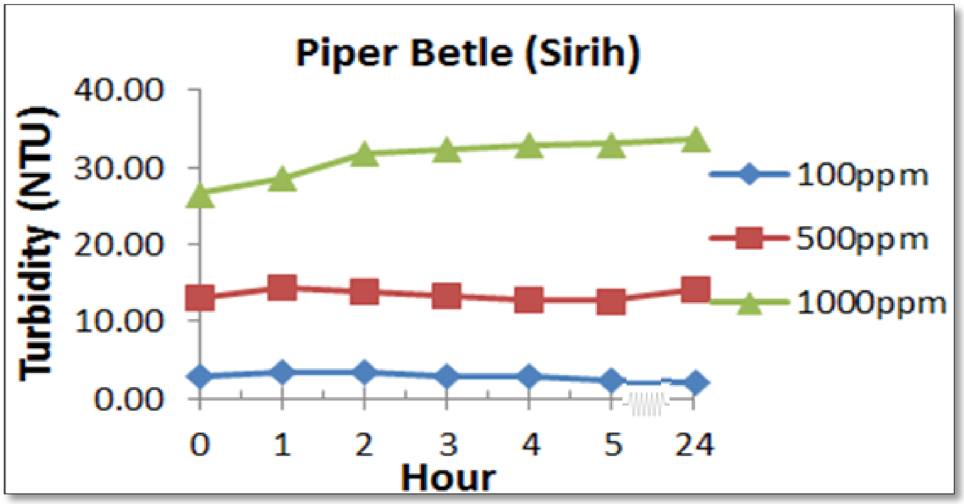


Figure 4.7 : Performance of Piper Betle at 100 ppm, 500 ppm and 1000 ppm tested with 100 ppm silicate solution

Piper Betle show same characteristic as two pervious samples, but at 1000 ppm concentration, the turbidity value increases unlike the curve in Camellia Sinensis and Premna cordifolia. Overall value are too large indicates there is lesser polyphenolic compounds amount in the extracted samples. The highest turbidity value gives reading of 33.67 NTU.

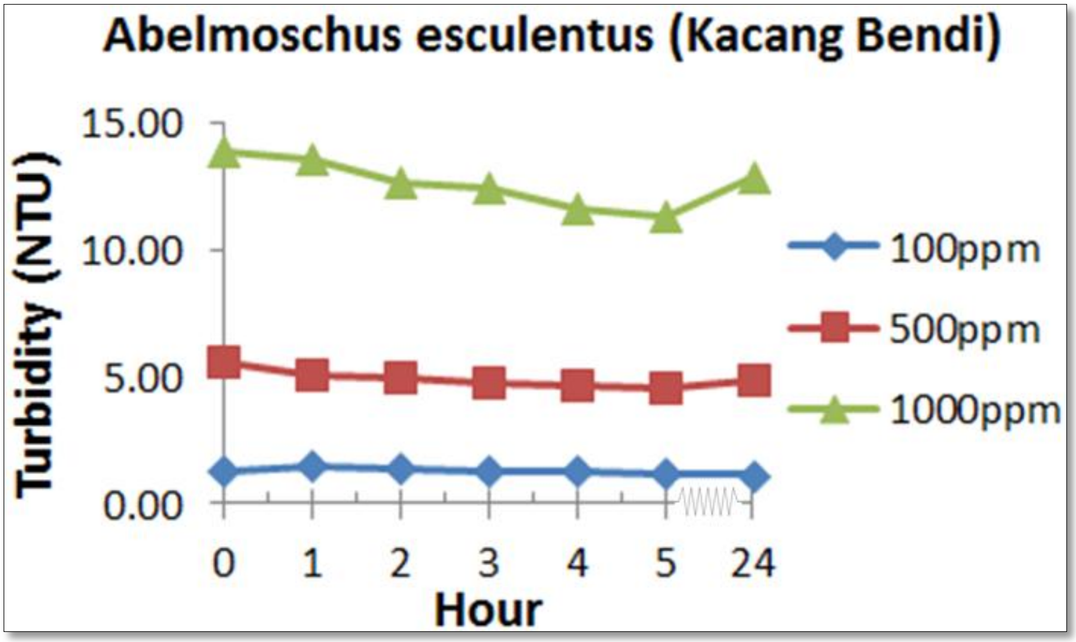


Figure 4.8 : Performance of Abelmoschus Esculentus at 100 ppm, 500 ppm and 1000 ppm tested with 100 ppm silicate solution

Abelmoschus esculentus show small decreasing value at low concentration of 100 ppm and 500 ppm. At 1000 ppm concentration, Abelmoschus esculentus showing decreasing turbidity which indicates the polyphenolic compounds forming complexes with the silicic acid or silicate in the solution. As the high turbidity values than Camellia sinensis are yield, this show that its performance is poor. This is because the cloudy green colour which affect the turbidity value during experiments.

The scale inhibitors were test in mixture of 50 ml of **200 ppm silicate solution** and 50 ml of synthetic water (brine).

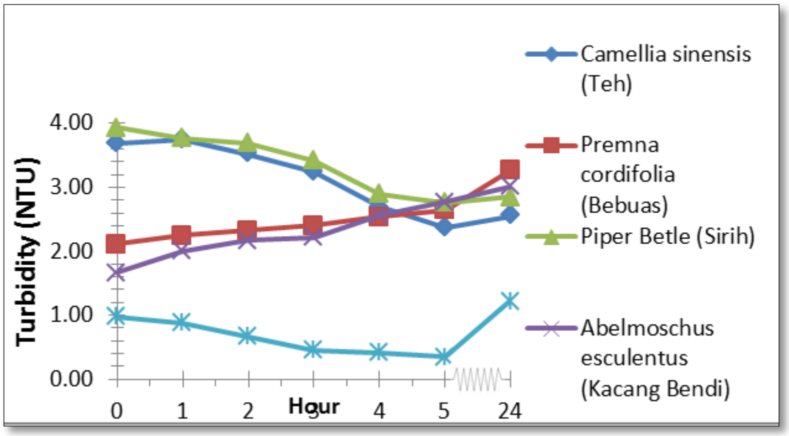


Figure 4.9 : Turbidity (NTU) of 100 ppm scale inhibitor concentration

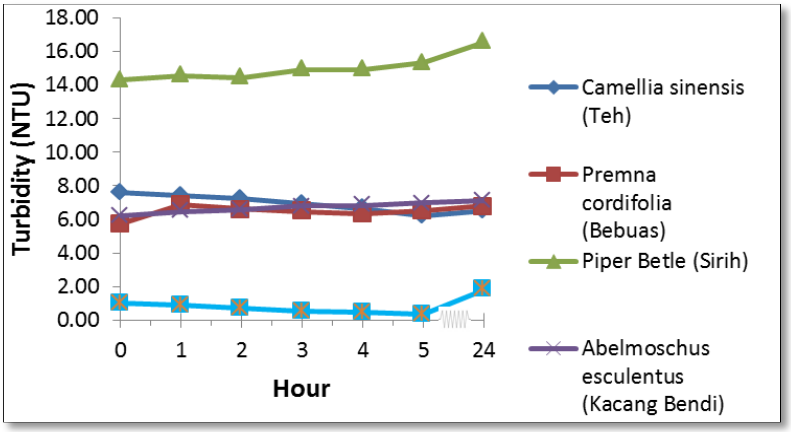


Figure 4.10 : Turbidity (NTU)) of 500 ppm scale inhibitor concentration

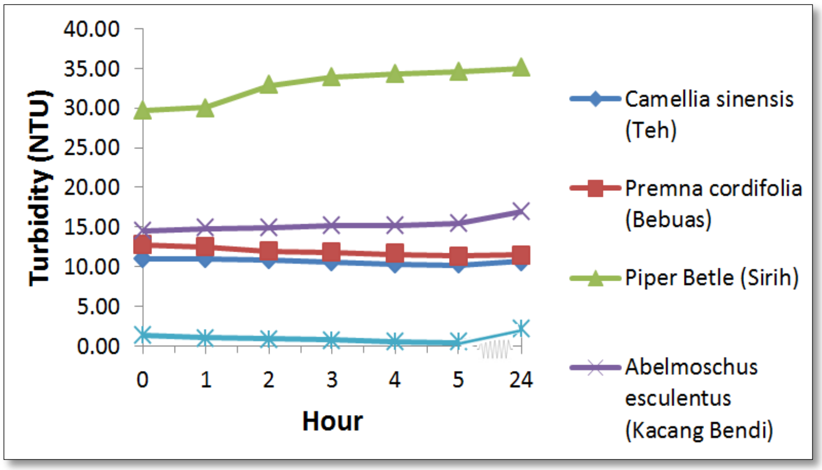
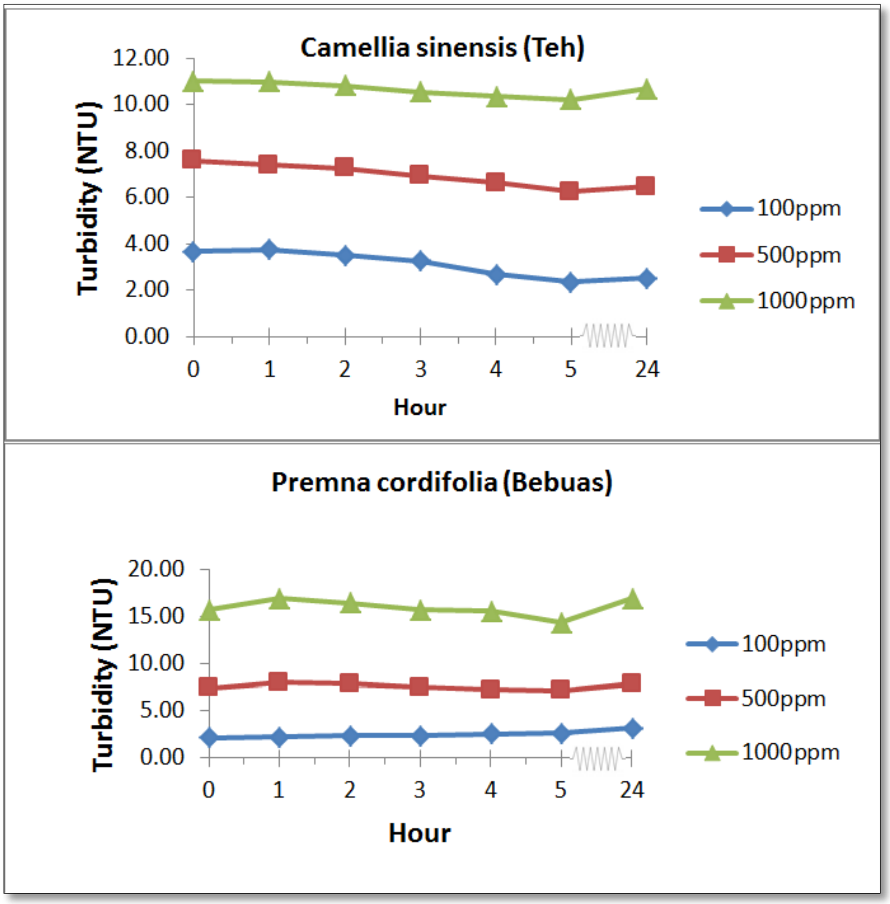


Figure 4.11 :Turbidity (NTU) of 1000 ppm scale inhibitor concentration

Note : The tabulated data can be referred at Appendix 12,13 and 14.

For the 200 ppm concentration silicate solution used, the details has been summarized for the four samples performance as follow:


Camellia sinensis (Teh)

Premna cordifolia (Bebuas)

Figure 4.12 : Performance of Camellia sinensis and Premna cordifolia at 100 ppm, 500 ppm and 1000 ppm tested with 200 ppm silicate solution

Both samples show similar decreasing value of turbidity when tested with 200ppm of silicate solution. The higher the concentration of the polyphenolic compounds will reduce more the turbidity value which also means help preventing the silicic acid or silicate ion to form compounds with metals ion especially magnesium and calcium ion. The lower the existence of compounds that combined with silicate ion or silicic acid, the lower the value of the turbidity.

The turbidity reading for both samples are higher than in 100 ppm silicate solution. The higher the concentration of the silicate ion used will yield higher turbidity reading. The only difference between both sample is Premna Cordifolia has generally higher reading of turbidity than Camellia Sinensis at 100 ppm, 500 ppm and 1000 ppm.

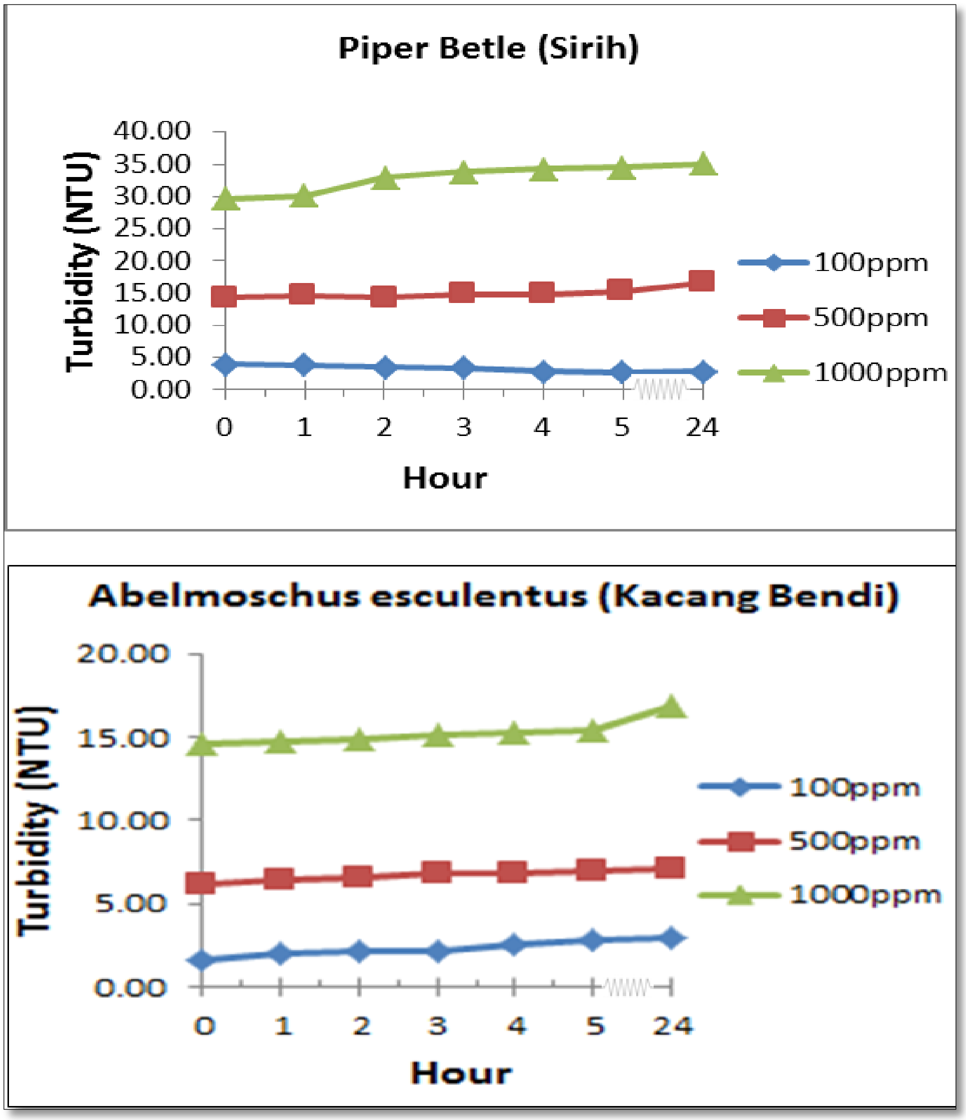


Figure 4.13 : Performance of Piper Betle and Abelmoschus esculentus (Kacang Bendi)

Both samples show increasing reading of turbidity. This increment can be explained by the concentration silicate solution. Due to high silicate concentration in the mixture tested, the polyphenolic compounds contained is not sufficient to form complexes with the high amount silicate ions or silicic acid. Thus the remaining silicic acid or silicate ions which are not forming complexes with polyphenolic compounds, formed compounds with other metal ions and eventually causing continuous increment of turbidity reading. Thus in order to overcome this problem, the higher concentration needed in order to inhibit silicate scales.

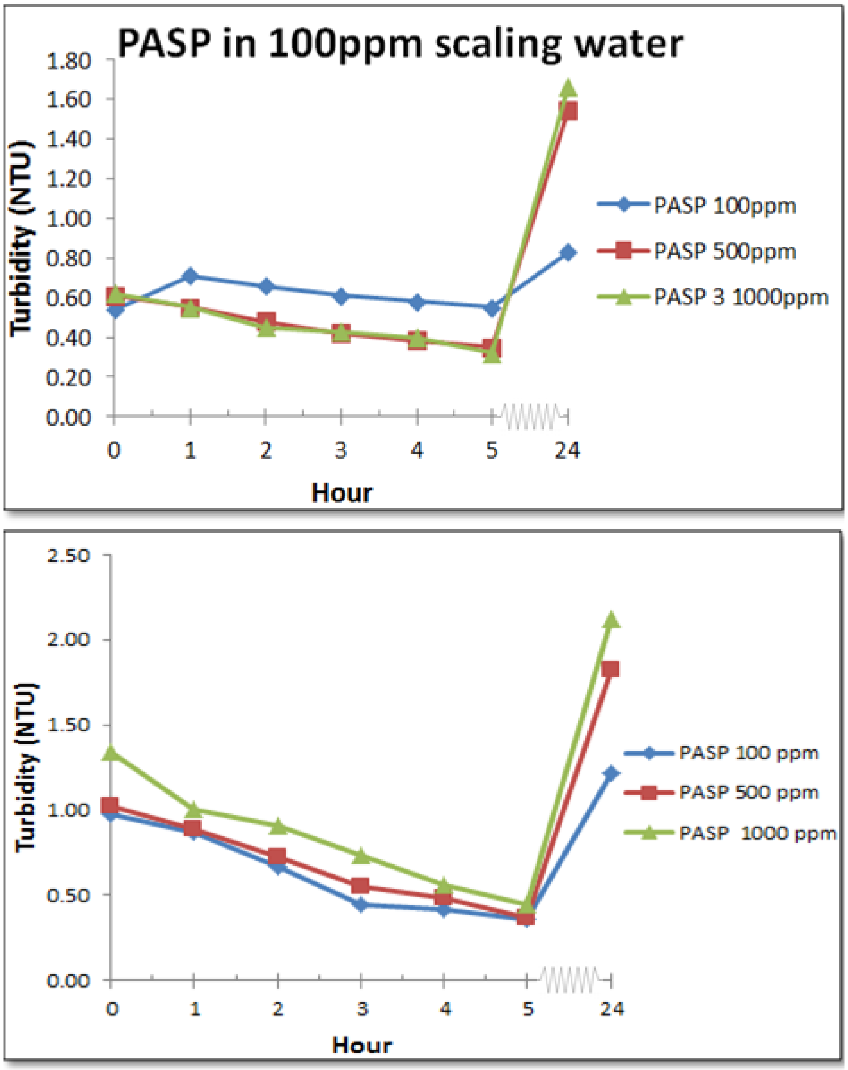


Figure 4.14 : Performance of PASP in both 100ppm and 200ppm scaling solution.

Note : The tabulated data can be referred at Appendix 8.

PASP performance in both respective scaling solution showing the decrease reading of turbidity which indicate the effectiveness of delaying silicate scale formation. As PASP know as chelating agent which form complexes with the silicate ions or silicic acid in the scaling solution. At high concentration of PASP (1000 ppm) the curve show steep curve that indicate the higher the concentration value yield lower turbidity reading.

Four candidate tested with the scaling solution are expected to yield result and trend curve same as PASP curve. As PASP displayed very high inhibition efficiency compare to four candidates because it is a commercialized scale inhibitor which present in industry. Its efficiency should be undeniably far better than other inhibitor tested.

There are others plant which has been studied for the same purposed which to exploit for becoming green inhibitor in oil and gas field application. The reasons to choose this plant instead of other rare or uncommon used plant are:

- Only the natural plant that has commercial value and commonly used had complete study of their chemical composition.
- Inadequate time and equipment to analyze the chemical composition of some plant which are uncommon to eat or not used yet in industries.
- Because they are grown worldwide and very abundant, thus the quantities to be used in making silicate scale inhibitor does not affect at all to the food industries or citizens requirement.

4.3 pH Test

pH Test has be conducted after the Turbidity Test on the four candidate at neutral condition. This test aims to analyze the pH effect on the result and simulating the condition of HF acidizing treatment on sandstone. As the pH Test consume more time, thus the promising candidate was only being tested with different pH value (acidic condition to emulating acidizing treatment condition).

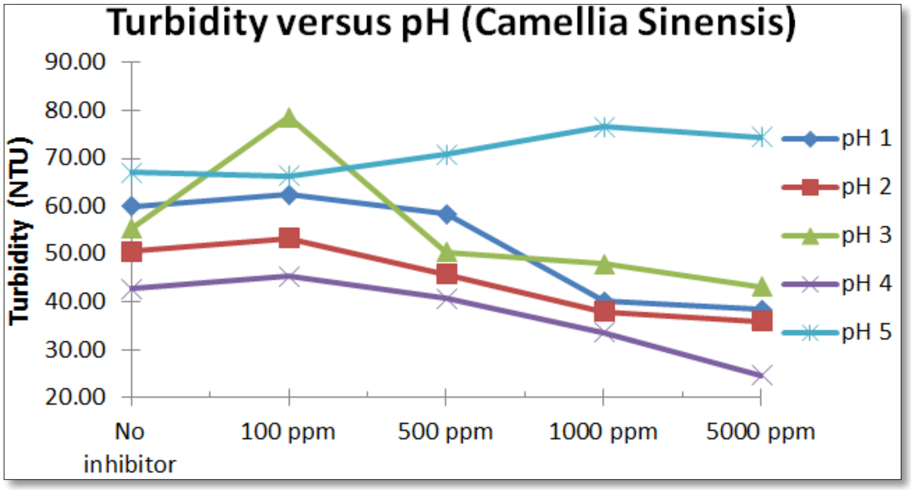


Figure 19 : Effect of pH on Camellia Sinensis performance

From the figure above, Camellia Sinensis shows decreasing curves of turbidity values which indicated the effectiveness of the green inhibitor to delay the polymerization and condensation of the silicic ion by forming Si-polyphenolic complexes. Higher turbidity indicates the high silicate compounds form in the scaling solution.

CHAPTER 5 : CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Premna cordifolia, Camellia sinensis, Piper Betle and *Abelmoschus esculentus* have been selected for the investigation of green silicate scale inhibitor. The four herbs contain polyphenolic compounds which have been identified as a new class of silica polymerization inhibitor (SPI). Polyphenolic compounds were reported can prevent the formation of silica during hydrofluoric acidizing treatment on sandstone. Through compatibility test, all four samples at 100 ppm, 500 ppm, 1000 ppm showing no precipitation when tested with mixtures of silicate solution and synthetic water (brine) indicating their compatibility. The four samples performance had been evaluated comparatively to conventional green scale inhibitor, Polyaspartate (PASP). 100 ppm, 500 ppm, 1000 ppm and 5000 ppm of PASP tested with the scaling solution as bench mark. Camellia Sinensis showed significance potential to inhibit the condensation and polymerization of silicic acid. The performance of Premna cordifolia, Abelmoschus esculentus and Piper Betle are not active as Camelia Sinensis. Turbidity of all brines added with green inhibitors showed higher turbidity values than that of brines added with PASP. It might be attributed to the high concentration of chlorophyll since the extracts were used in their pure forms without other treatments.

In conclusion, polyphenolic compounds from Camellia Sinensis in this project could be promising organic silicate scale inhibitor which acts as silica polymerization inhibitor (SPI). Further researches and effective extraction procedures on Camellia sinensis and Premna cordifolia are needed to further explore their potential.

1.2 Recommendations

This project has a huge potential for green silicate scale inhibitor investigation can be improved further if the right amount of time, resources and equipment is allocated. There are several recommendations that can be considered in order to enhance and improve the project to obtain much better outcome.

1. The need of precise experimental procedures to extract completely the polyphenolic compounds amount contained in the four candidates in order to yield optimistic result. The method used in removing green colour in this project are not 100% effectives.

2. The green colour from chlorophyll in the four samples should be completely removed by advance experimental procedure. The green colour will impaired the result and also lead to misinterpretation of data in turbidity test and spectrophotometric test.

3. The amount of the four candidates must be in large quantity so that we can obtain more polyphenolic compounds amount in order to be tested on higher concentration as the real concentration of silicate ion or silicic acid in the sandstone formation would be comparatively high during HF acidizing treatment on sandstone.

4. UTP should provide more than one equipment such as XRF (X-ray fluorescence) or practice good maintenance of equipment. The broke down of equipment causing the test of performance on scaling solution with the inhibitor to be cancelled. If the test can be conducted, there will be more supporting data which can be yield. The XRD machine performance has been dropped as due to some technical problem, thus the result is not so accurate and precise.

5. Good acquisition of chemicals or reagents will improve further the project research quality. Silica reagent for silicomolybdate method in spectrophotometric test are estimated to delivered more than 10 weeks as the only one company does not having stock for the present time. Very diluted

hydrofluoric acid for simulation of HF acidizing treatment condition also are not allowed to be acquired from UTP chemical laboratory as due to safety factors.

6. Besides Static Test, Dynamic Tube Blocking Test or Core flooding should also carry out to measure the performance of green inhibitor in high temperature and high pressure condition. As there are too many students queuing to use the equipment, there is no time to yield some experiment result on this type of test as the test consumed about three weeks per student to be carried out.

7. There are some unpredicted readings which impact the overall result. Turbidity meter sometimes showing unnatural reading, thus repetition of the testing should be applied. The author taking the average from 3 repetitive reading for the result as the time constraint. In order to obtain more accurate result, the whole turbidity test set should be carried out twice.

References.

[1] Alicia B. , How to Remove Chlorophyll From Leaves Retrieves October 25, 2013, from http://www.ehow.com/how_5789711_remove-chlorophyll-leaves.html

[2] Amjad Z. and R. W. Zuhl Lubrizol(2008). An Evaluation of Silica Scale Control Additives for Industrial Water System .Corrosion Conference and Expo 2008, NACE International. No 08368.

[3] Ashraf E.A Basbar, Khaled A.Elraies and Reza E.Osgouei(2013) "Formation Silicate Scale Inhibition during Alkaline Flooding : Static Model " SPE 164669

[4] Arensdorf, J., Kerr, S.,Miner,K., and Ellis-Toddington,T. (2011). Mitigating Silicate Scale in Production Wells in an Oilfield in Alberta. Paper SPE 141422, SPE International Symposium on Oilfield Chemistry ,Woodlands, Texas, USA, 11-13 April 2011.

[5] Bazin B., N. K. (2004) A New Class of Green Mineral Scale Inhibitors for Squeeze Treatments Society of Petroleum Engineering (pp.1-12). United States of America.

[6] Crowe, C.W .(1986).Precipitation of Hydrated Silica from Spent Hydrofluoric Acid: How Much a Problem Is It? JPT38(11): 1234-1240.doi: 10.2118/13083-PA

[7] Darrell L. Gallup, and Charles J. Hinrichsen, Chevron Energy Technology Company (2008). Control of Silicate Scales in Steam Flood Operations," Society of Petroleum Engineers Journal, SPE 114042 , pp1-11

[8] David Hasson (2011). State of the Art of Friendly "Green" Scale Control Inhibitors: A Review Article. I&EC Research, 7601-7067.

[9] Gdanski, R.D (1994) Fluorosilicate Solubilities Impact HF Acid Compositions. SPE Prod. & Facilities 9 (4): 225-229.doi : 10.2118/27404-PA

[10] Gdanski, R.D (1997a). Kinetics of Primary Reaction of HF on Alumino-Silicates.Paper SPE 37459 presented at the Productions and Operations Symposium, Oklahoma,USA 9-11 March, doi: 10.2118/37459-MS

[11] Gdanski, R.D (1997b) Kinetics of Secondary Reaction of HF on Alumino-Silicates. Paper SPE37214 presented at International Symposium on Oildfield Chemistry, Houston , Texas, USA18-21 Februar, doi: 10.2118/37214-MS

[12] Gdanski, R.D (1999). Kinetics of Secondary Reaction of HF on Alumino-Silicates. Paper SPE31076 presented at the International Symposium on Formation Damage Control , Lafayette Louisiana, USA, 14-15 February, doi:10.2118/31076-PA

[13] Jasbir S. Gill, Ph.D. and Gregory T. Jacobs. (2011). Development of New Silica Inhibitor – Laboratory and Field Study. GRC Transactions, Vol. 35

[14] Jia Q., Zhou B., Zhang R., Chen Z., (2002) Daqing Oilfield Construction Design and Research Institute, and Zhou Yuchun, Daqing Oilfield Natural Gas Company. “Development and Application of a Silicate Scale Inhibitor for ASP Flooding Production Scale” Society of Petroleum Engineers Journal. January SPE 74675

[15] Joseph A. Scot K , Kirk M. and Baker Hughes Incorporated (2011). Mitigating Silicate scale in Production Wells in an Oilfield in Alberta. Society of Petroleum Engineers Journal . April 2011 .SPE 141422.

[16] Kashpura V.N, Potapov V.V.(January 2000) .Study of the Amorphous Silica Scales Formation at the Mutnovskoe Hydrothermal Field (Russia) Sgp-Tr-165.

[17] Kell and Malcolm. (2009). Scale Control Production Chemicals for the Oil and Gas Industry. CRC Press.

[18] Konstantinos D. Demadis and Mualla Ö. (2009). Inhibitory Effects of “Green” Additives On The Crystal Growth Of Sparingly Soluble Salts. ISBN: 978-1-60692-092-3. pp. 265-287

[19] Kostas D. D, Aggeliki S and Antonia K (2007). Control Of Colloidal Silica: Can Chemical Additives Untie The "Gordianknot" Of Scale Formation? Corrosion Conference and Expo 2007, NACE International. No 07058

[20] Lieu, V.T. and Miller, S.G. (1985) .A Chemical Reactions with Reservoir Sand in the Recovery of Petroleum by Alkaline Flooding,” Society of Petroleum Engineers Journal, August, 1985, pp587-593

[21] Mazen A. Ateyyat, M. A. (2009) Aqueous extracts of some medicinal plants are as toxic as Imidacloprid to the sweet potato whitefly, Bemisia tabaci. Journal of Insect Sceince, 2

[22] Maria T. Escribano-Bailon and Celestino Santos-Buelga. “Polyphenol extraction from foods.” Retrieved on 10 October 2013 from http://en.wikipedia.org/wiki/Polyphenol#cite_note-68

[23] Mohd Shukri, C. Alan and A.R. Site Noorzuraini (2011) Polyphenols and Antioxidant Activities of Selected Traditional Vegetables” pp. 69-83.

[24] Nawrocki, Jacek , (1997) The silanol group and its role in liquid chromatography Journal of Chromatography, volume 779,29-72. Retrieve from http://www. sciencedirect. com/ science/article /pii/S0021967397004792

[25] Norbert K, Brigitte B., Alain Z.(2004) Institut Francais du Petrole; Green Inhibitors for Squeeze Treatments: A Promising Alternative NACE International, ID 04537

[26] Pittaco. A (2012). How does pH affects the rate of chemical reaction? Retrieves 25 10, 2013, from WikiAnswer: http://wiki.answers.com/Q/How_does_pH_affects_the_rate_of_chemical_ reaction

[27] Pokrovski, G. and Schoot J. (1998) Experimental Study of the Complexation of Silicon and Germanium with Aqueous Organic Species : Implication for Germanium and SiliconTransport and Ge/Si Ratio in Natural Water. Geochim. Cosmochim. Acta 62: 3.413-3428.

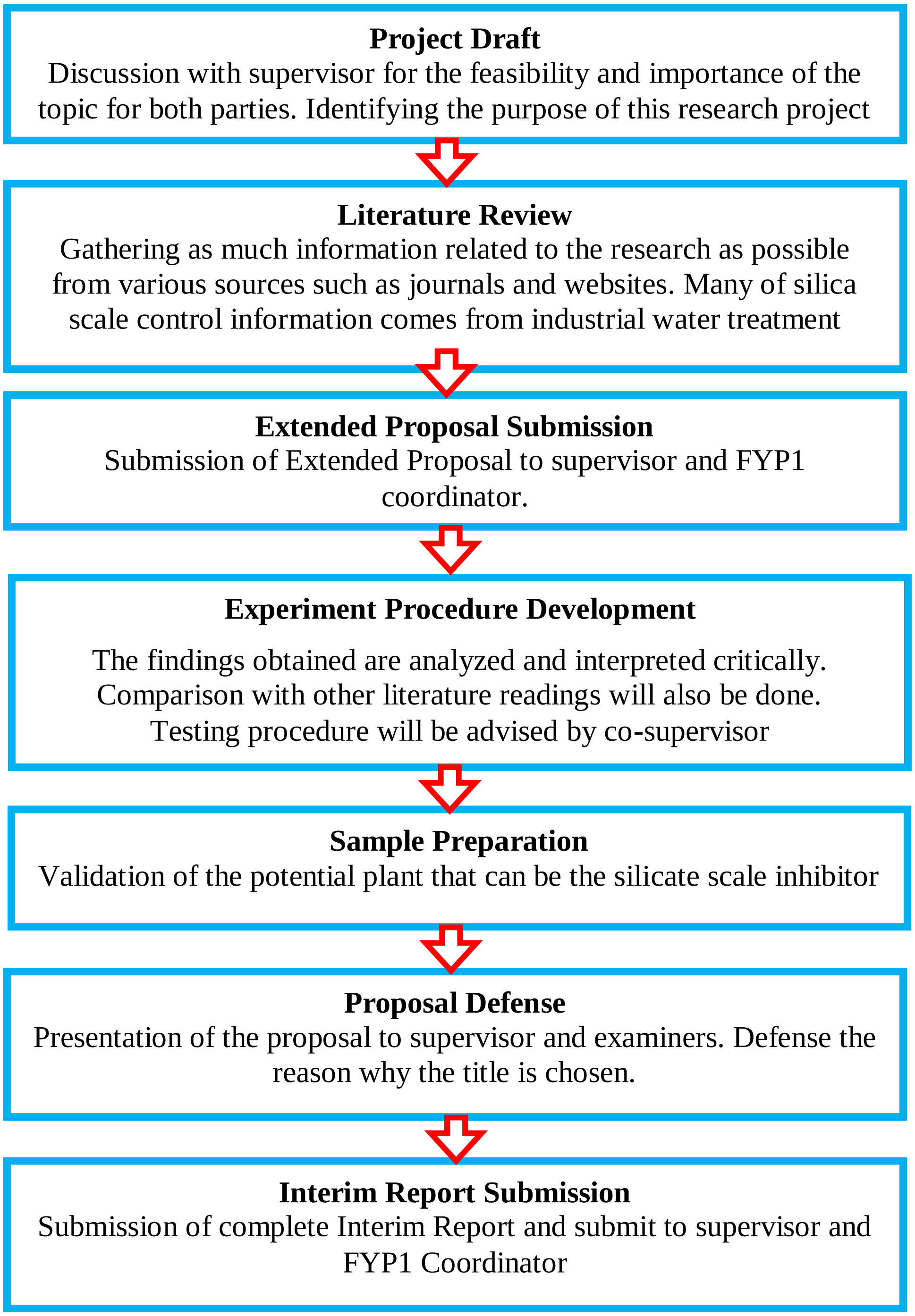
[28] Reyes-Garcia E. (2011). Application and Laboratory Studies of a New Class of Silica Polymerization of Bio-renewable Origin". SPE 143663

[29] Robert J. Ross, Kim L., Donlar Corp, James E. Shannon,(1996) .Global SOS, Polyaspartate Scale Inhibitors-Biodegradable Alternatives to Polyacrylates, NACE International, ID 96162

[30] Thomas R.L., H.A. Nasr-El-Din, S. Mehta, V. Hilab, J.D. Lynn, (2002) Saudi Arabian Oil Company: “The Impact of HCl to HF Ratio on Hydrated Silica Formation During the Acidizing of a High Temperature Sandstone Gas Reservoir in Saudi Arabia”. SPE Annual Technical Conference and Exhibition, 29 September-2 October 2002, San Antonio, Texas, doi: 10.2118/77370-MS

APPENDICES

Appendix 1 : Several key milestones for this research project in FYP 1 in **first semester** must be achieved in order to meet the objective of this project and FYP1 requirement .



Project Draft
Discussion with supervisor for the feasibility and importance of the topic for both parties. Identifying the purpose of this research project

Literature Review
Gathering as much information related to the research as possible from various sources such as journals and websites. Many of silica scale control information comes from industrial water treatment

Extended Proposal Submission
Submission of Extended Proposal to supervisor and FYP1 coordinator.

Experiment Procedure Development

The findings obtained are analyzed and interpreted critically. Comparison with other literature readings will also be done. Testing procedure will be advised by co-supervisor

Sample Preparation
Validation of the potential plant that can be the silicate scale inhibitor

Proposal Defense
Presentation of the proposal to supervisor and examiners. Defense the reason why the title is chosen.

Interim Report Submission
Submission of complete Interim Report and submit to supervisor and FYP1 Coordinator

Appendix 2 :For the **second semester** several key milestones for this research project in FYP2

Lab Experiment and Data Acquisition
Conducting all test which has been planned on the four sample in order to investigate the inhibition performance.

Result Analysis and Discussion
Collecting the result in orderly mannered, analyze and discussed how the experiments and variables affect the result yielded.

Submission of Progress Report
Submission of all the progress work which has been done .

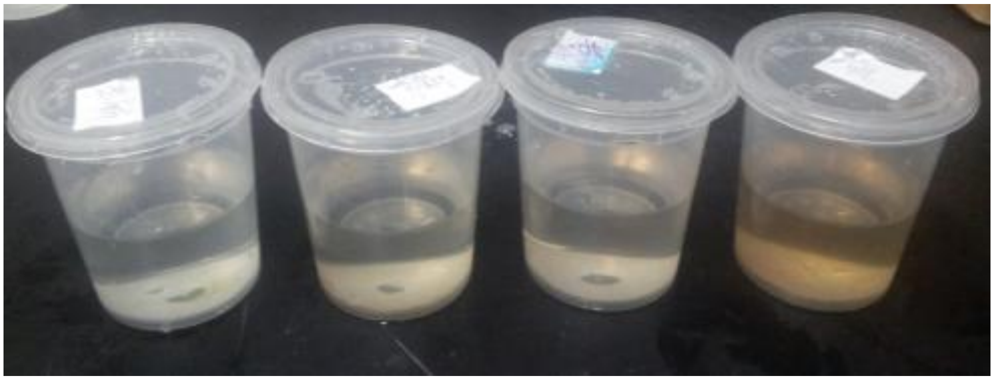
Pre-SEDEX

Prepare presentation of the progress works before qualified to SEDEX

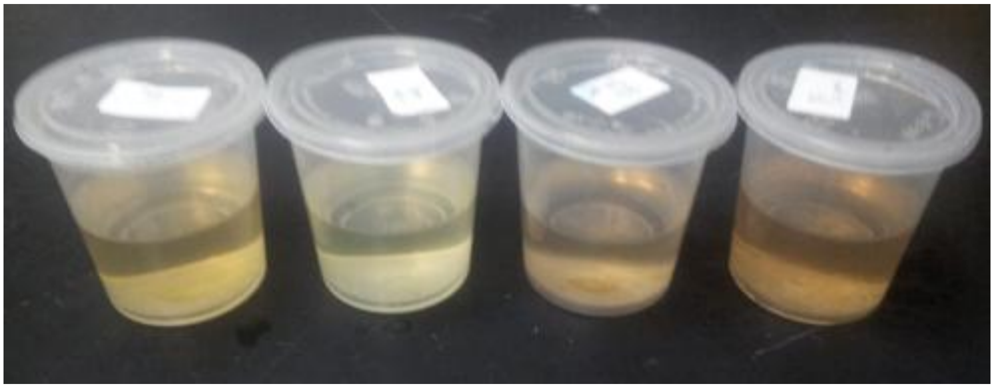
Submission of Draft Report

Submission of Technical Paper & Dissertation (softbound)
Prepare Technical Paper about the research study and submit Dissertation (softbound) to FYP Coordinator

Oral presentation & Submission of Dissertation (hardbound)
Submission of complete Dissertation and submit to supervisor and FYP Coordinator



Appendix 3 : Mixture 100 ppm concentration of four saMples extracted being tested



Appendix 4 : Mixture 500 ppm concentration of four samples extracted being tested

Appendix 5 : Mixture 1000 ppm concentration of four samples extracted being tested

Appendix 6 : 100ppm , 500ppm, 1000ppm and 5000 ppm of PASP being tested

Appendix 7 : Turbidity value of different PASP concentration

	Turbidity (NTU)				
Duration (hour)	No inhibitor	100 ppm	500 ppm	1000 ppm	5000 ppm
0	58.54	55.56	40.33	20.55	9.21
1	78.52	76.66	60.52	35.46	9.88
2	94.83	83.44	68.33	40.67	10.33
3	101.52	94.80	70.10	53.71	11.67
4	104.33	98.40	75.45	61.02	14.33
5	105.46	101.65	80.66	68.33	16.78
24	106.23	103.66	85.45	76.46	20.45

Appendix 8 : Turbidity reading (NTU) of in both 100 ppm and 200ppm scaling solution

	100ppm scaling solution			200 scaling solution		
Duration (hour)	PASP 100ppm	PASP 500ppm	PASP 1000ppm	PASP 100ppm	PASP 500ppm	PASP 1000ppm
0	0.54	0.61	0.62	0.98	1.02	1.34
1	0.71	0.55	0.55	0.87	0.89	1.01
2	0.66	0.48	0.45	0.67	0.73	0.91
3	0.61	0.42	0.43	0.45	0.55	0.74
4	0.58	0.38	0.40	0.42	0.48	0.56
5	0.55	0.35	0.32	0.36	0.37	0.45
24	0.83	1.54	1.66	1.22	1.82	2.12

Appendix 9,10,11 are data of the scale inhibitors were test in mixture of 50 ml of **100 ppm silicate solution** and 50 ml of synthetic water (brine) .

Appendix 9 :Turbidity reading (NTU) of 100 ppm scale inhibitor concentration

Duration (hour)	Camellia sinensis (Teh)	Premna cordifolia (Bebuas)	Piper Betle (Sirih)	Abelmoschus esculentus (Kacang Bendi)	PASP
0	1.25	1.71	3.00	1.29	0.54
1	1.31	1.78	3.54	1.50	0.71
2	1.37	1.84	3.47	1.38	0.66
3	1.42	1.96	3.03	1.30	0.61
4	1.40	2.24	2.82	1.25	0.58
5	1.37	2.46	2.33	1.20	0.55
24	1.38	3.01	2.07	1.12	0.83

Appendix 10 : Turbidity reading (NTU) of 500 ppm scale inhibitor concentration

Duration (hour)	Camellia sinensis (Teh)	Premna cordifolia (Bebuas)	Piper Betle (Sirih)	Abelmoschus esculentus (Kacang Bendi)	PASP
0	4.09	6.50	13.00	5.54	0.61
1	4.60	7.76	14.45	5.06	0.55
2	4.40	6.94	13.86	4.96	0.48
3	4.21	6.83	13.34	4.77	0.42
4	4.14	6.79	12.90	4.68	0.38
5	4.04	6.65	12.70	4.56	0.35
24	4.23	6.79	14.05	4.88	1.54

Appendix 11 : Turbidity reading (NTU) of 1000 ppm scale inhibitor concentration

Duration (hour)	Camellia sinensis (Teh)	Premna cordifolia (Bebuas)	Piper Betle (Sirih)	Abelmoschus esculentus (Kacang Bendi)	PASP
0	6.01	14.70	26.60	13.80	0.62
1	8.80	15.80	28.58	13.56	0.55
2	8.45	15.32	31.70	12.65	0.45
3	7.69	14.80	32.40	12.42	0.43
4	7.45	14.34	32.80	11.60	0.40
5	7.29	13.90	33.01	11.30	0.32
24	7.95	14.70	33.67	12.79	1.96

Appendix 12, 13 and 14 are data of the scale inhibitors were test in mixture of 50 ml of **200 ppm silicate solution** and 50 ml of synthetic water (brine) .

Appendix 12: Turbidity reading (NTU) of 100 ppm scale inhibitor concentration

Duration (hour)	Camellia sinensis (Teh)	Premna cordifolia (Bebuas)	Piper Betle (Sirih)	Abelmoschus esculentus (Kacang Bendi)	PASP
0	3.68	2.12	3.93	1.67	0.98
1	3.74	2.25	3.76	2.00	0.87
2	3.52	2.33	3.69	2.17	0.67
3	3.24	2.41	3.42	2.22	0.45
4	2.69	2.54	2.90	2.56	0.42
5	2.37	2.63	2.76	2.77	0.36
24	2.55	3.26	2.85	3.01	1.22

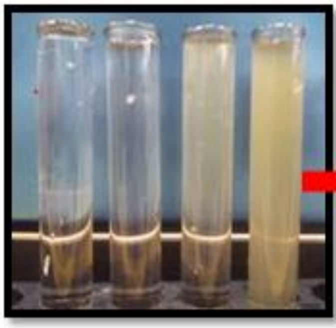
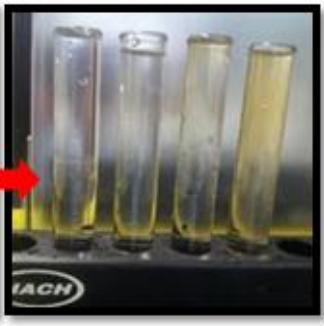
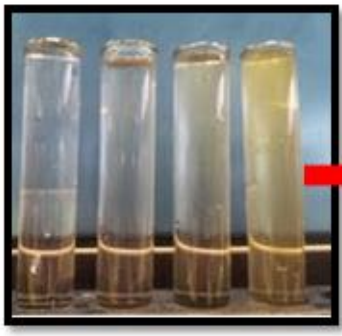
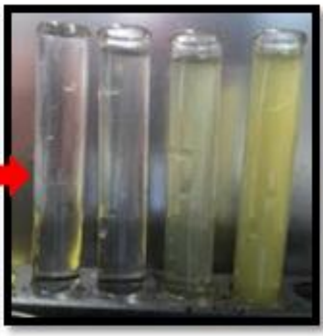
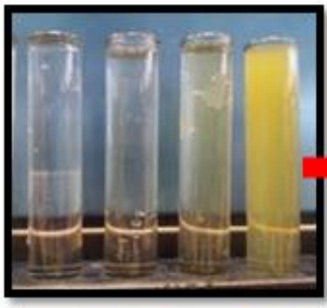
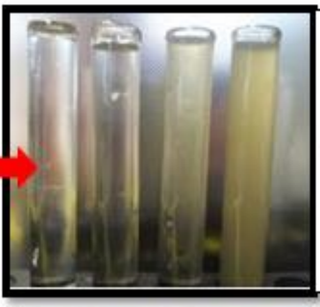
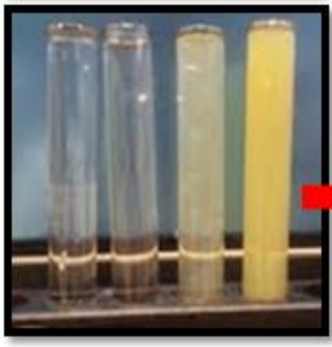
Appendix 13: Turbidity reading (NTU) of 500 ppm scale inhibitor concentration

Duration (hour)	Camellia sinensis (Teh)	Premna cordifolia (Bebuas)	Piper Betle (Sirih)	Abelmoschus esculentus (Kacang Bendi)	PASP
0	7.59	5.74	14.32	6.23	1.02
1	7.40	6.89	14.56	6.44	0.89
2	7.25	6.60	14.44	6.57	0.73
3	6.93	6.47	14.89	6.79	0.55
4	6.65	6.32	14.91	6.83	0.48
5	6.23	6.48	15.34	6.96	0.37
24	6.48	6.78	16.55	7.12	1.82

Appendix 14: Turbidity reading (NTU) of 1000 ppm scale inhibitor concentration

Duration (hour)	Camellia sinensis (Teh)	Premna cordifolia (Bebuas)	Piper Betle (Sirih)	Abelmoschus esculentus (Kacang Bendi)	PASP
0	11.01	12.80	29.71	14.56	1.34
1	10.98	12.45	30.08	14.77	1.01
2	10.77	11.96	32.87	14.93	0.91
3	10.54	11.78	33.96	15.14	0.74
4	10.35	11.56	34.25	15.24	0.56
5	10.22	11.33	34.54	15.45	0.45
24	10.66	11.45	35.01	16.89	2.12

Appendix 15 : Visual observation for the compatibility test.

Before	After	Descriptions
		**Camellia sinensis (Teh)** 100ppm, 500 ppm, 1000 ppm and 5000ppm of samples in 250 ml test tube initially at room temperature (left) and final observation after 24 hours left in oven at 90 ˚ Celsius.
		**Premna cordifolia (Bebuas)** 100ppm, 500 ppm, 1000 ppm and 5000 ppm of samples in 250 ml test tube initially at room temperature (left) and final observation after 24 hours left in oven at 90˚ Celsius.
		**Piper Betle (Sirih)** 100ppm, 500 ppm, 1000 ppm and 5000ppm of samples in 250 ml test tube initially at room temperature (left) and final observation after 24 hours left in oven at 90 ˚ Celsius.
		**Abelmoschus esculentus (Kacang Bendi)** 100ppm, 500 ppm, 1000 ppm and 5000 ppm of samples in 250 ml test tube initially at room temperature (left) and final observation after 24 hours left in oven at 90 ˚ Celsius.