Parametric Study of Soxhlet Extraction of Phenolic Compounds from *Vernonia amygdalina* Leaves

Oluwaseun Ruth Alara
Centre of Excellence for Advanced Research in Fluid Flow (CARIFF),
Faculty of Chemical and Natural Resources Engineering,
Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300, Gambang, Pahang, Malaysia
ruthoalao@gmail.com

Nour Hamid Abdurahman
Centre of Excellence for Advanced Research in Fluid Flow (CARIFF),
Faculty of Chemical and Natural Resources Engineering,
Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300, Gambang, Pahang, Malaysia

Siti Kholijah Abdul Mudalip
Faculty of Chemical and Natural Resources Engineering,
Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300, Gambang, Pahang, Malaysia

Chinonso Ishmael Ukaegbu
Faculty of Industrial Sciences and Technology,
Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300, Gambang, Pahang, Malaysia

Nour Hamid Azhari
Faculty of Pure and Applied Sciences,
International University of Africa, Khartoum, Sudan

Nassereldeen Ahmed Kabbashi
Bioenvironmental Engineering Research Centre (BERC),
Department of Biotechnology Engineering (BTE),
Kulliyyah of Engineering (KOE), International Islamic University Malaysia,
Gombak, 50728, Kuala Lumpur, Malaysia

Abstract—Phenolic compounds are important secondary metabolites with different pharmacological activities. The effects of extraction parameters of Soxhlet extraction method which include extraction time, solid-to-solvent ratio and ethanol concentration on the recoveries of extraction yields, total phenolic content (TPC) and total flavonoid content (TFC) from extracts of *Vernonia amygdalina* leaves were investigated in this study using one-factor-at-a-time experiment (OFAT). Then, the functional group characterization of the extracts at maximum conditions was carried out. The results obtained showed that the three considered extraction parameters significantly influence the recoveries of extraction yield, TPC and TFC. Highest recoveries of extraction yield (14.22±0.19% w/w), TPC (75.94±2.15 mg GAE/g d.w.) and TFC (46.53±1.14 mg QE/g d.w.) were obtained at extraction time of 2 h, solid-to-solvent ratio of 1:20 g/mL and ethanol concentration of 60% v/v. Moreover,

six major peaks were identified from the Soxhlet extraction of *V. amygdalina* leaves at maximum conditions using Fourier transform infrared spectroscopy (FTIR) analysis. Thus, this study showed that *V. amygdalina* leaf is a potential source of phenolic compounds which can be influence by the extraction parameters.

Keywords—Vernonia amygdalina; Soxhlet extraction; Total phenolic content; Total flavonoid content

1. INTRODUCTION

Phenolic compounds are secondary metabolites that exhibit a different range of physiological activities such as anti-inflammatory, antioxidant, anti-allergenic, anti-thrombotic, anti-diabetic, vasodilatory, and cardioprotective effects [1]. Reports had shown that higher consumption of beverages, vegetables and fruits increase the intake of phenolic compounds [2]. Besides the health benefits, phenolic compounds provide protection against predators and pathogens, contribute towards the sensory and colour characteristics of vegetables and fruits [3].

Vernonia amygdalina is commonly known as "bitter leaves" in English due to its bitter taste. Also, it is referred to as ironweed with a soft-wooded shrub that can regenerate rapidly. The extracts from this plant had been reported to possess several pharmacological activities such as antibacterial, anti-diabetic, anti-allergic, anti-fungi, anticancer, anti-inflammatory, anti-leukaemia, analgesic, antipyretic, and antioxidant [4]. Several extraction methods which include traditional and unconventional are used in the extraction of phenolic compounds from plant materials. Soxhlet extraction method had been used to extract bioactive compounds from *V. amygdalina* leaves using ethyl acetate and n-hexane as extracting solvents whereby the extracts exhibited antibiotic activity [5]. This method had been used to extract bioactive compounds from other plant materials [6, 7]. Adama et al. had used the Soxhlet method to extract substitute for hops from *V. amygdalina* leaves. The phytochemical analysis showed that the sample extracts had brewing properties: total resin (20.4%), iso-alpha (8.52%), essential oil (1.20%), fat contents (7.00%), and alpha acid of 9.27% [8]. However, the studies did not report the effect of each Soxhlet extraction parameters on the yield of extract, total phenolic content and total flavonoid content in *V. amygdalina* leaf using ethanol as the extracting solvent. Thus, this study employed Soxhlet extraction method to investigate the effects of extraction parameters which include extraction time, solid-to-solvent ratio and ethanol concentration on the recoveries of extraction yields, total phenolic content (TPC) and total flavonoid content (TFC) from the extracts of *V. amygdalina* leaves using one-factor-at-a-time experiment (OFAT). The functional group characterization of the extracts at maximum condition was as well evaluated.

2. MATERIALS AND METHODS

A. Plant Sample and procurement of chemicals

Fresh samples of *V. amygdalina* leaves were obtained from Gambang, Malaysia. The leaves were washed in tap water and dried at room temperature until constant weight was reached. Afterwards, the dried leaves were ground using a blender, sieved to an average particle size of 0.105 mm and stored in an airtight dark container prior to the Soxhlet extraction process.

The following chemicals and reagents were purchased from Sigma Aldrich (M) Sdn Bhd, Selangor: Methanol (99.9% purity), ethanol (99.5% purity), quercetin, gallic acid, Folin-Ciocalteu reagent, aluminium chloride salt, and sodium carbonate anhydrous. The distilled water was obtained from the Faculty of Chemical and Natural Resources Engineering laboratory.

B. Soxhlet extraction of V. amygdalina leaves

The plant sample (10 g) was weighed and loaded inside a thimble of Soxhlet extractor. A volume of aqueous ethanol based on the experimental design was measured into a 250-mL round bottom flask (Table 1). The extractor was placed on a heating mantle. The experimental process kick-started by randomly varying the extraction time as 1, 2, 3, and 4 h (Table 1). As the solvent was being heated using a mantle heater, at the boiling temperature, it began to evaporate by moving through the apparatus to the condenser. The condensate dripped into the reservoir containing the thimble with plant matrix. Once the level of solvent reached the siphon, the solvent containing extract refluxed back into the round bottom flask and the recycling began again. After the extraction time has been reached, the extract was allowed to cool down to room temperature, filtered and concentrated to dryness using a rotary evaporator. Then, the extraction yield, TPC and TFC were calculated. The experimental process was carried out in triplicate. The yield of extracts of *V. amygdalina* leaves was evaluated as follows:

$$\%Yield\ of\ extracts = \frac{Weight\ of\ extracts\ \ from\ plant\ sample\ (w)}{Weight\ of\ dried\ \ plant\ powder\ (w)} * 100\% \quad (1)$$

Table 1: Randomly selected experimental design for the Soxhlet extraction of *V. amygdalina* leaf using OFAT.

Extraction time (h)	**F:S (g/mL)**	**Ethanol concentration (% v/v)**
1	1:10	20
2	1:15	40
3	1:20	60
4	1:25	80

C. Evaluation of total phenolic content

Folin-Ciocalteu (FC) colourimetric assay was used to evaluate the content of phenolics in the extract from *V. amygdalina* leaves using Soxhlet extraction technique. The reported method by Alara et al. was employed for the assay [9]. In brief, the extract was re-dissolved in aqueous ethanol to make a concentration of 5 g/L. Then, 0.2 mL of FC was mixed with 1000 µL of the extract. A 600 µL of 0.2 mM Na_2CO_3 solution was then added to the mixture after 5 min of leaving it to incubate at room temperature. Afterwards, the mixture was left for 2 h in the dark and the absorbance was recorded at 765 nm against the blank (sample solvent) using a UV-vis Spectrophotometer (Hitachi U-1800, Japan). Gallic acid was used as the standard at concentration of 50-500 mg/L ($y = 0.0006x + 0.0169$, $R^2 = 0.9903$) and the analysis was carried out thrice. The results were given as milligram gallic acid equivalent per gram dry weight (mg GAE/g d.w.) as shown in Equation (2).

$$TPC = \frac{c * V}{m} \quad (2)$$

where c is the sample concentration from the calibration curve (mg/L), V is volume (L) of solvent used in the extraction, and m represents the weight (g) of the dried sample used.

D. Evaluation of total flavonoid content

The method described by Alara et al. was used to evaluate the total flavonoid in the extract from *V. amygdalina* leaves [9]. In brief, the extract was re-dissolved in aqueous ethanol to make a concentration of 1 g/L. Afterwards, 1 mL of extract was thoroughly mixed with 1 mL of $AlCl_3$ solution. The absorbance of the mixture was recorded at 420 nm after leaving it for 60 min at room temperature using a UV-VIS Spectrophotometer (Hitachi U-1800, Japan). Quercetin was used as the standard at concentration of 50-500 mg/L ($y = 0.112x + 0.178$, $R^2 = 0.9945$) and the analysis was carried out thrice. The results were given as milligram quercetin equivalent per gram dry weight (mg QE/g d.w.) as shown in Equation (3).

$$TFC = \frac{c * V}{m} \quad (3)$$

where c is the sample concentration from the calibration curve (mg/L), V is volume (L) of solvent used in the extraction, and m represents the weight (g) of the dried sample used.

E. Functional characterization of the extract

The functional groups in the dried extract from *V. amygdalina* leaves were identified using Fourier transform infrared (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany) equipped with OMNIC software. The spectra were scanned from wave number between 4000 and 500 cm^{-1} with a resolution of 4 cm^{-1} [10]. The spectra obtained for the extracts were interpreted with a chart for characteristics infrared absorption frequencies of functional groups.

3. RESULTS AND DISCUSSION

Figures 1 to 3 illustrate the effects of individual extraction factors, namely, extraction time, solid-to-solvent ratio and ethanol concentration on the recoveries of extraction yields, TPC and TFC from extracts of *V. amygdalina* leaves. The influences of each factor are discussed in the followed subsections.

A. Influence of extraction time on the recovery yields

The effect of extraction time was studied by varying time between 1 to 4 h at the fixed solid-to-solvent ratio of 1:10 g/mL and ethanol concentration of 20% v/v as presented in Figure 1. The yields increased as the extraction time increased from 1 to 2 h, however, the yields tends to reduce as the extraction time was increased beyond 2 h. This might be attributed to the degradation of phenolic compounds due to longer exposure to localized heating [11]. Thus, the extraction time of 2 h was used to investigate the effects of other parameters.

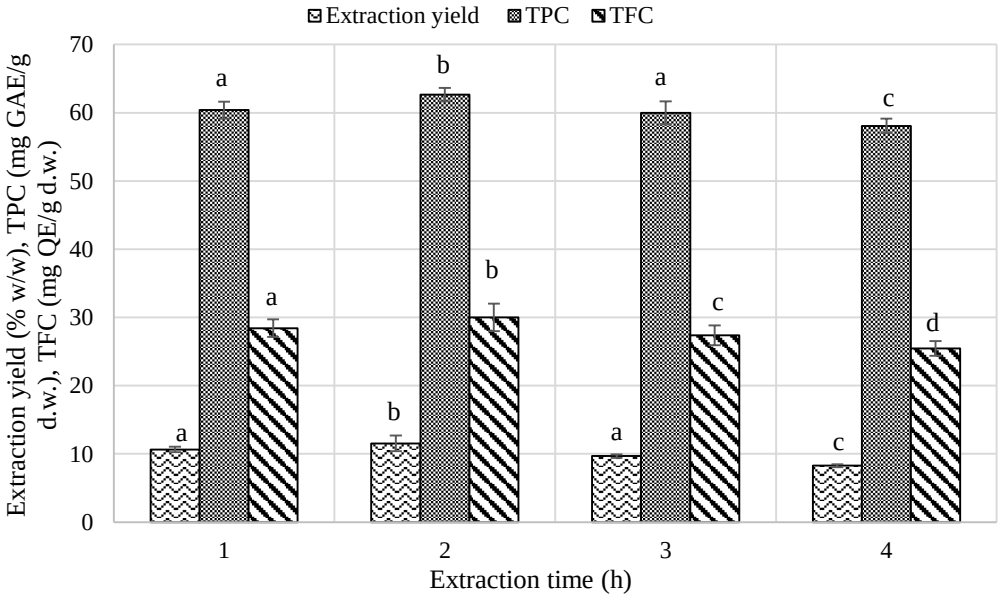

Figure 1: Influence of extraction time on the yields of extract, TPC and TFC from *V. amygdalina* leaves.

Different letters along the column indicate a significant difference ($p < 0.05$).

B. Influence of solute-to-solvent ratio on the recovery yields

The solute-to-solvent ratio is another important parameter that influences the yields from plant sample using Soxhlet extraction method. At fixed extraction time of 2 h and ethanol concentration of 20% v/v, the solid-to-solvent ratio was varied as 1:10, 1:15, 1:20, and 1:25 g/mL. The results showed that solid-to-solvent ratio of 1:20 g/mL allows the best extraction of yield and phenolic compounds (Figure 2). This can be correlated to the mass transfer principles, whereby the driving force for mass transfer in the extraction process is appraised to be the solute concentration gradient between solid and solvent used [12]. Therefore, the solid-to-solvent ratio of 1:20 g/mL was considered to study the effect of ethanol concentration on the recovery yields.

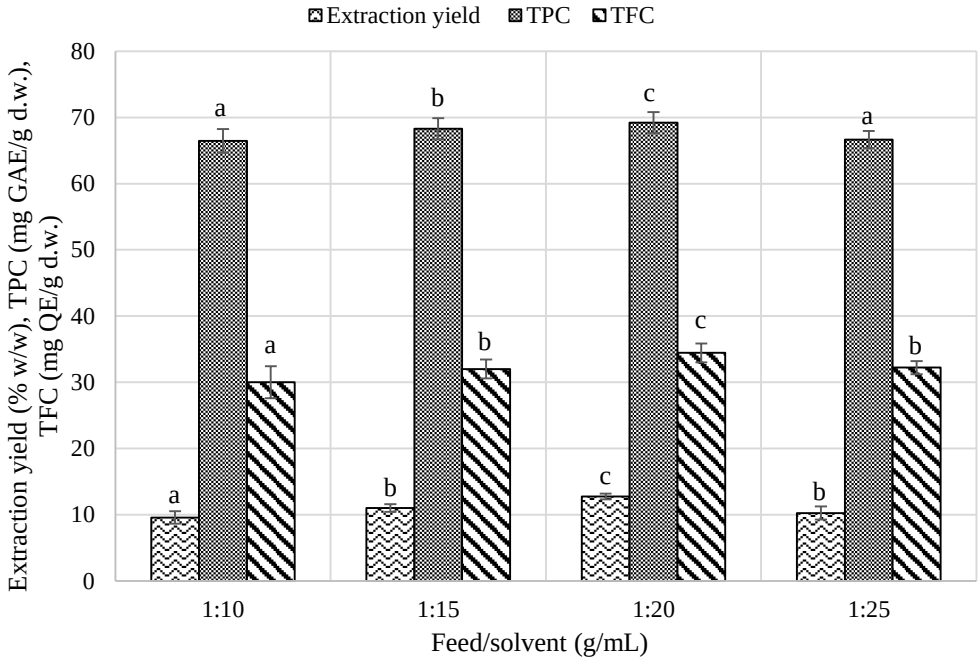

Figure 2: Influence of solid-to-solvent ratio on the yields of extract, TPC and TFC from *V. amygdalina* leaves. Different letters along the column indicate a significant difference ($p < 0.05$).

C. Influence of ethanol concentration on the recovery yields

Furthermore, the effect of ethanol concentration varied as 20, 40, 60, and 80% v/v at constant extraction time of 2 h and a solid- to-solvent ratio of 1:20 g/mL. As illustrated in Figure 3, the recovery yields improve as the concentration increases from 20 to 60% v/v, further increase in ethanol concentration caused a decline in the yields of extracts, TPC and TFC from *V. amygdalina* leaves. Studies had shown that binary-solvent system is more efficient than mono-solvent in the extraction of phenolic compounds from the plant matrix [12]. It has been reported that the presence of some amount of water can aid the mass transfer process in increasing the relative polarity of the solvent, thus enhance its solubilizing ability through effective swelling of the plant matrix. Therefore, the surface area for solute-solvent interaction increases [13]. Thus, the addition of water to ethanol enhance extraction efficiency of phenolic compounds as illustrated in Figure 3. Therefore, the highest recoveries of extract, TPC and TFC from *V. amygdalina* leaves were 14.22±0.19% w/w, 75.94±2.15 mg GAE/g d.w. and 46.53±1.14 mg QE/g d.w., respectively at extraction time of 2 h, solid-to-solvent ratio of 1:20 g/mL and ethanol concentration of 60% v/v.

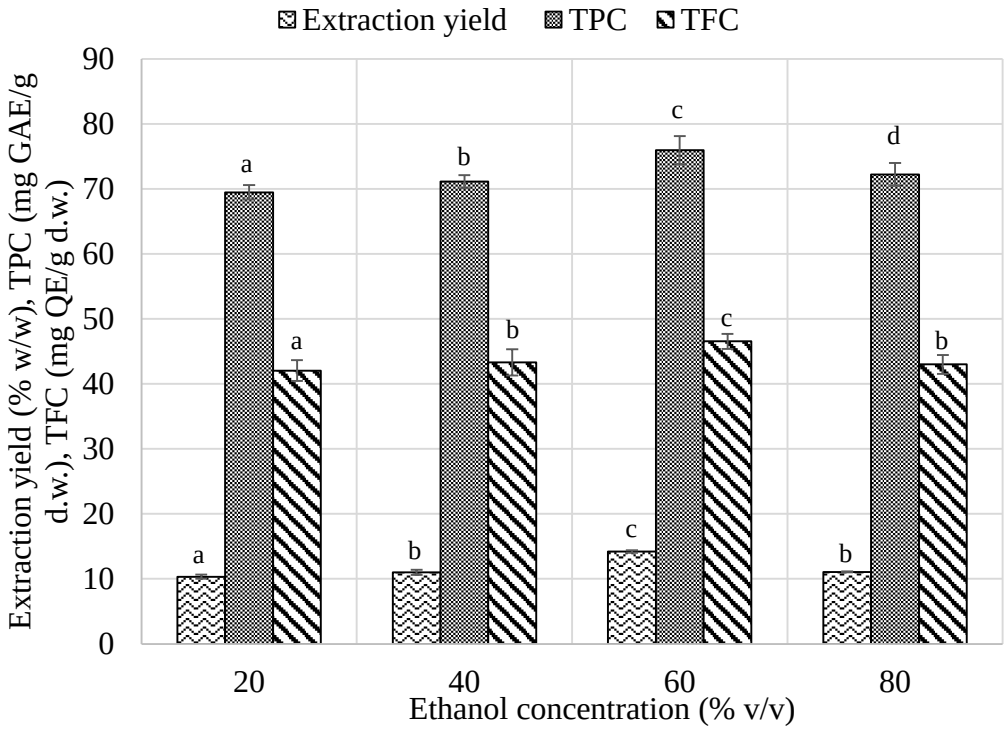

Figure 3: Influence of ethanol concentration on the yields of extract, TPC and TFC from *V. amygdalina* leaves. Different letters along the column indicate a significant difference ($p < 0.05$).

D. Characterization of the extracts using FTIR analysis

The infrared spectra obtained at the highest conditions of Soxhlet extraction method for recoveries of TPC and TFC from extracts of *V. amygdalina* leaves is illustrated in Figure 4. Six major peaks were identified from the extract of *V. amygdalina* leaves. The stretch absorption band at 1065.59 cm^{-1} and 1104.88 cm^{-1} reveal the presence of alcohols group C-O, the presence of amines stretching absorption band at 1632.81 cm^{-1}, the presence of C-H absorption bands was observed at 2849.29 cm^{-1} and 2917.11 cm^{-1}, the presence of broad absorption band at 3328.50 cm^{-1} shows the presence of O-H stretching vibration of water molecules. This strongly suggested the presence of phenolic compounds in the extract. Soxhlet spectra show similar peaks as reported by Zakaria et al. [14].

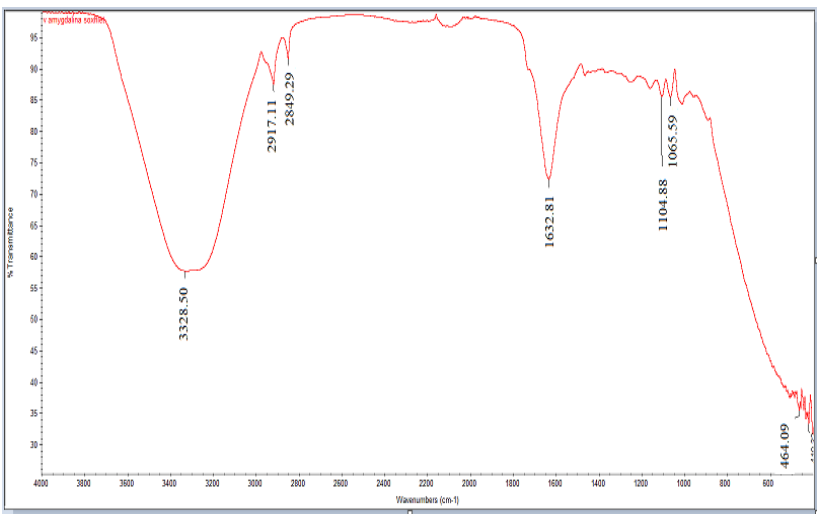

Figure 4: The identified functional groups in extract from *V. amygdalina* leaves.

4. CONCLUSION

The influences of extraction time, solid-to-solvent ratio and ethanol concentration on the recoveries of extraction yields, TPC and TFC from the extracts of *V. amygdalina* leaves using Soxhlet extraction method had been evaluated in this study. The results showed that the highest recoveries of extraction yield, TPC and TFC were 14.22±0.19% w/w, 75.94±2.15 mg GAE/g d.w. and 46.53±1.14 mg QE/g d.w., respectively at extraction time of 2 h, solid-to-solvent ratio of 1:20 g/mL and ethanol concentration of 60% v/v. However, six major peaks were identified from the Soxhlet extraction of *V. amygdalina* leaves at maximum conditions using Fourier transform infrared spectroscopy (FTIR) analysis.

ACKNOWLEDGMENT

The authors of this manuscript appreciate the financial support of Universiti Malaysia Pahang under the grant number RDU180329.

REFERENCES

[1] Singh, G. Singh, S. C. Sati, and S. Sachdeva, "An overview on manufacturing of lubricating greases and testing processes," Int. Conf. Adv. Res. Innov., pp. 43–6, 2014.

[2] J. Wright, "Grease Basics," Noria, 2008.

[3] Donley, Handbook of Advances in Additive Lubricants and Grease technology, Auris Reference, 2012.

[4] M. A. A. Mastor, "Negara dibanjiri minyak hitam" in Utusan Malaysia, pp.7, December 2011.

[5] T. Bhaskar, M. A. Uddin, A. Muto, Y. Sakata, Y. Omura, K. Kimura, and Y. Kawakami, "Recycling of waste lubricant oil into chemical feedstock or fuel oil over supported iron oxide catalysts," Fuel, vol. 83, pp. 9–15, 2004.

[6] GVS, "Lubricants Market Analysis By Product, Industrial (Process Oils, General Industrial Oils, Metal Working Fluids, Industrial Engine Oils), Automotive (Heavy-Duty Engine Oils, Hydraulic & Transmission Fluid, Gear Oil, Passenger Vehicle Engine Oils, Automatic)," in Grand View Research, 2016.

[7] EPA, "Managing, reusing, and recycling used oil," United States Environmental Protection Agency, 2017.

[8] TN50, "Trend: environment" in TN50, 2017.

[9] S. Hegazi, "Conversion of used oil into lubricating grease and characterictic evaluation," Int. J. Sci. Res., vol. 4, pp. 1894–1898, 2015.

[10] K. Ebisike, B. E. A. Daniel, M. N. Anakaa, H. M. Kefas, and S. O. O. Olusunie, "Effect of sodium hydroxide thickener on grease production," Am. Chem. Sci. J., vol. 13, pp. 1–8, 2016.

[11] M. N. Razali, M. A. A. Aziz, W. N. A. W. M. Hamdan, N. A. M. Salehan, and R. M. Yunus, "Synthesis of grease from waste oils and red gypsum," Aus. J. Basic & Appl. Sci., vol. 11, pp. 154–159, 2017.

[12] T. M. Verdura, G. Brunette, and R. Shah, Lubricating Greases Fuels and Lubricants Handbook: Technology, Properties, Performance, and Testing, ASTM International, pp. 225, 2003.

[13] IMOA, "Molybdenum-sulfur compounds in lubrication," IMOA, 2007.

[14] Iheme, J. C. Offurum, and F. O. Chukwuma, "Production and blending of sodium based water-resistant lubricating greases from petroleum and petrochemical by-products," Am. J. Comput. Sci. Eng. Surv., vol. 2, pp. 70–8, 2014.

[15] P. M. Lugt, Grease Lubrication in Rolling Bearings, Chichester: John Wiley & Sons, Inc., 2013.

[16] Advancepetro, "Different types of Brake Fluid," 2009.

[17] M. Pirro, M. Webster, and E. Daschner, Lubrication Fundamentals, 3rd ed., CRC Press, 2016.

[18] L. G. Ludwig, "Storing grease to avoid bleed and separation," in Machinery Lubrication, February 2012.

[19] A. Rezasoltani, and M. M. Khonsari, "On monitoring physical and chemical degradation and life estimation models for lubricating greases," Lubricants, vol. 4, pp. 24, 2016.

[20] Doyle, "General grease overview and bearing lubrication," ALS Tribol.,2015.