Development of Hand Sanitizer Enhanced with Green Coffee Beans Extract

Muhamad Nazmi Bin Ahmad and Dr. Ummi Kalthum Ibrahim

Faculty of Chemical Engineering, Universiti Teknologi Mara

***Abstract*— This research focused on Chlorogenic acids from green Arabica coffee beans extract Green coffee beans extract application is still low due to lack of research on its potential and benefits. Since green coffee beans claim to be rich in Chlorogenic acids, it will influence the potential benefits of the extract. Chlorogenic acids also has been proven to exhibit antibacterial activity on pathogenic bacteria. In response to utilize the ability of Chlorogenic acids from green coffee beans, this study conducted to develop sanitizer enhanced with green coffee beans extract which can be an alternative formulation compared to alcohol based. Green coffee beans extract was extracted by using Soxhlet extraction method and Chlorogenic acids was analyses by using UV-Vis spectroscopy. Central Composite design of response surface methodology (RSM) with 13 experimental runs was used to investigate the optimum conditions for the extraction, and the selected variables were solvent volume from 100mL to 300mL and extraction temperature from 100 ºC to 200 ºC. The maximum predicted Chlorogenic acids concentration (0.012 mg/mL) was obtained at the solvent volume and extraction temperature of 300mL and 200ºC respectively. Thus, the result from this extraction has been utilized for the formulation of hand sanitizer with green coffee beans extract. Evaluation of developed hand sanitizer was conducted by using disc diffusion method on the Escherichia Coli. An effective zone inhibition (13mm) of hand sanitizer was obtained when using green coffee beans extract at 300mL solvent volume and 200°C extraction temperature.**

Keywords*— Coffee, GCB, CGA, UV-Vis spectroscopy, Soxhlet extraction, antimicrobial

I. INTRODUCTION

Infections outbreak in today community much more related to lack of good hygiene practice where people are more susceptible to numerous microorganisms. Infections can mostly transmit with unclean or soiled hands especially children where transmission comes when playing in microorganisms contaminated area or contaminated toys. Without cleaning their hands, children sometimes will put their fingers into their mouth or having meal without proper hand washing practice [1]. Not only children are susceptible to infections, healthcare workers also can be threatening from various pathogens transmitted from hospital setting, equipment and infected patients [1].

Preventive action should be taken to avoid from being infected by microorganism where it is suggested to practice proper hand cleaning or using sanitizer [2]. Most practical sanitizer that commonly being use is hand sanitizer as it convenient, portable and easy to use when we are doing daily activity or working. Common formulation of hand sanitizer will consist of 60 to 95 per cent alcohol as active ingredient to kills microorganism. Research has proven that alcohol can kills bacteria by inhibiting their sporulation and spore germination [3]. Besides alcohol, antimicrobial agent also can be retrieved from plant where there is wide phytochemical content that believed to be useful for drugs formulation [4].

Naturally, plant exhibits their own protection or natural defense against animals, insect and also microorganism due to their chemical compounds which can be varies according specific plants itself [4]. Research also stated that phytochemicals present in plant with complex structures and biologically active compounds [4]. This phytochemical initiate scientist for further research on newer compounds derived from plants for treatment of infections. Therefore, research on plant derivative has been accelerated in recent years in order to develop natural formulation on antimicrobial agent. During the age before pharmaceutical drug were developed, the ancestor found healing power of plant to cure any illness and infections.

Continuous research has found that many plants rich in various antimicrobial agents such as alkaloids and flavonoids. Recent studies have discovered antimicrobial property contributed by Chlorogenic acids (CGA) which are uniquely a plant derivative chemical [5]. This CGA can be found in green coffee bean (GCB) which the most abundant of all acids in coffee. Contrast to roasted coffee bean, CGA break down during roasting and thus reducing the valuable content of the acids itself [6]. Investigation has proven that CGA effectively increase the permeability of cell membrane against pathogen which reduces its barrier function. Reducing of pathogen cells barrier function will lead to cell death [5]. This finding will assist researcher to construct and formulate antimicrobial agent from GCB extract in order to produce a safer product for consumer.

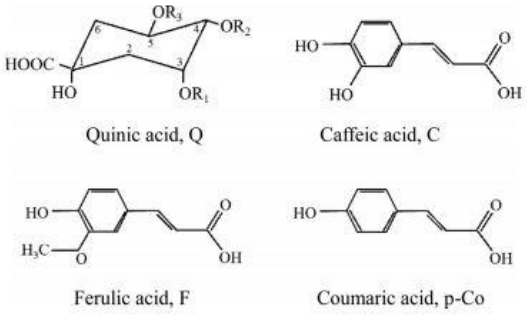

Fig. 1: Composition and molecular structure of Chlorogenic acids [7]

II. METHODOLOGY

A. Materials and sample preparation

Arabica coffee beans bought from F Marketing (Selangor, Malaysia) were used for further extraction process in this research. The coffee beans quality (no defect) were selected and sorted manually for screening. Any black or defected coffee beans were

rejected. GCB has been grounded into form of fine powder to ease (increasing the total surface area) the extraction process. Fine powder of GCB was stored in airtight container to avoid from being exposed to air. Ethanol as solvent for Soxhlet extraction were bought from Chemiz (Selangor, Malaysia). Glycerine were bought from EmC^2 Technology (Selangor, Malaysia). Carbomer were bought from Take It Global (Pulau Pinang, Malaysia).

B. Experimental design

The experiment was designed using Design Expert version 11, where a Central Composite Design experimental design was employed in order to optimize the extraction of GCB. The experiment was designed on two factors, two response that generated 13 experimental runs. The two independent factors are extraction temperature, and solvent volume. The summary of this is as shown in Table 1.

Table 1: Summary of the experimental design

Factor	Name	Unit	Low	High
A	Temperature	℃	100	200
B	Solvent volume	mL	100	300

C. Extraction of green coffee bean extract

GCB extraction were performed using Soxhlet extraction process referring to previous extraction method performed by Redfern, Verran, Burdass and Kinninmonth [8]. Soxhlet extraction apparatus were set up according to laboratory standard operation procedure. 25g of fine GCB were used to fill the thimble inside the Soxhlet extractor. Ethanol were used as solvent as it is recommended by previous research Oliveira, Da Silva, Santos and Queiroz to extract phenolic compound from GCB [9]. The ethanol has been added into round bottom flask where volume of ethanol was manipulated ranging from 131mL to 329mL. Round bottom flask was heated with heating plate where ethanol going to evaporate through the Soxhlet extractor. Temperature of extraction was manipulated from 79.29°C to 220.71°C. Vapour of ethanol that passes through condenser of Soxhlet extractor was condense and drops into thimble compartment. Ethanol condensate that reaches the siphon level was flowing back into round bottom flask where cycle of evaporation to condensation of ethanol happens. Time of extraction were fixed for 360 minutes for each extraction. After 6 hours of extraction, mixture of ethanol and GCB extract were evaporated using Heodolph rotary evaporator (519-71310-00) to obtain only GCB extract.

D. Chlorogenic acid analysis

GCB extract were undergone UV-Vis spectroscopy analysis to characterize and quantify the concentration of CGA resulting after Soxhlet extraction. This characterization method was adapted with slightly modification by previous research conducted by Belay and Gholap [10] and Navarra, et al. [11]. Characterization of CGA were conducted by using Lambda 750 (Perking Elmer) UV-Vis spectroscopy where the maximum wavelength of CGA is at 324nm as stated by Belay and Gholap [10]. GCB extract were diluted with ethanol at ratio 1:9 and poured into cuvette for analysis. Absorbance value were recorded in order to compare the value with standard CGA calibration curve as shown in Figure 2 given by Navarra, et al. [10].

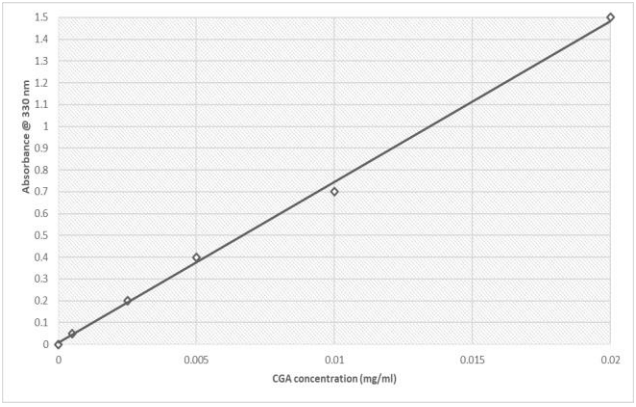

Fig. 2: Absorbance values of CGA at 330nm of wavelength as a function of the solution concentrations

The equation of CGA calibration curve was interpreted by equation (1) where value of absorbance from UV-Vis analysis were substituted into the equation to obtain the value of CGA concentration.

$$y = 71.086x + 0.0152 \quad (1)$$

E. Formulation of hand sanitizer enhanced with green coffee bean extract

Formulation method were carried out with slightly modification of Balkrishna, et al. [12] and Ningsih, Zusfahair, Kartika and Fatoni [13] method of hand sanitizer formulation. Formulation were conducted under room temperature and pressure. Ingredients were measured according to referred amount before undergo mixing process. As presented in Table 1, mixing process were aided by stirring action of magnetic stirrer. Deionized water, GCB extract and Glycerine were mixed together followed by slow addition of Carbomer to avoid formation of clumps. Stirring action were conducted continuously until the mixture is well mixed.

Table 2: Hand sanitizer formulation

Item No.	Ingredient	Form	Purpose
1	Deionized Water	Liquid	Base liquid
2	Carbomer	Powder	Thickener
3	Glycerine	Liquid	Humectants
4	Green coffee beans extract	Liquid	Antibacterial agent

Table 3: Amount of hand sanitizer formulation ingredient

Formulation		Amount
Item No.	Ingredient	
1	Deionized Water	50 mL
2	Carbomer	0.2g
3	Glycerine	0.5 mL
4	Green coffee extract	1 mL

F. Antimicrobial analysis

Antimicrobial analysis on the formulation of hand sanitizer were conducted by disc diffusion method. Agar plates were contaminated with *Escherichia Coli* (*E. Coli*) as the preparation of disc analysis. The antibiotic assay disc is then being enriched with formulation of hand sanitizer containing GCB extract. The disc was introduced into contaminated agar plate and incubated at 35.8 °C for 24 hours. Diameter of inhibitory zones were measured after incubation [14].

G. Optimization studies using response surface methodology

Response surface methodology has been used to study the optimization of extraction temperature, solvent volume resulting to CGA concentration and zone inhibition responses. Thus, response surface methodology was used in this study to investigate the optimum process parameters for the extraction of GCB using Soxhlet extraction. The factors considered are solvent volume and extraction temperature for optimal extraction with high concentration of CGA. A two factors, two response Central Composite Design (CCD) was employed using Design Expert 11 software to examine the optimum conditions of GCB extraction using Soxhlet extraction. The generated runs for the CCD investigated in this work consist of 13 experimental runs.

III. RESULTS AND DISCUSSION

A. Result of CGA concentration from the extraction of GCB

Extraction of green coffee beans has been conducted successfully where Soxhlet extraction method are able to obtain extract with various saturation of yellowish coloured extract. These various in colour of extract was strongly affected by the temperature and solvent volume during extraction and also could be an indication of the content of the extract itself. Therefore, it also can be seen from Table 4 where the CGA concentration are different according to its extraction condition.

Table 4: Results of Soxhlet extraction

Run	Temperature (°C)	Solvent volume (mL)	CGA concentration (mg/mL)
1	100	160	0.0049
2	200	160	0.0084
3	100	300	0.0077
4	200	300	0.0182
5	79.29	230	0.0021
6	220.71	230	0.0058
7	150	131	0.0072
8	150	329	0.0062
9	150	230	0.0142
10	150	230	0.007
11	150	230	0.0069
12	150	230	0.0052
13	150	230	0.0076

Table 5: ANOVA for Linear model (CGA concentration)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0001	2	0	2.15	0.1669	not significant
A-Temperature	0	1	0	3.22	0.1031	
B-Solvent volume	0	1	0	1.09	0.3214	
Residual	0.0001	10	0			
Lack of Fit	0.0001	6	0	1.31	0.4147	not significant
Pure Error	0	4	0			
Cor Total	0.0002	12				

From the Analysis of variance (ANOVA) for linear model as shown in Table 4.2, it can be seen that the F-value from the model gives out value of 2.15 indicating that the model is not significant due to the noise. The p-value from the ANOVA give out the value of 0.1669 which means that there is a change of the F-value is large occurring due to the noise at about 16.69%. However, the P-value that less than 0.05 could indicates a significant model term. For this linear model terms, it indicates that the are no significant model terms since it is greater than 0.1. It may require model reduction in order to improve this model since it has an insignificant model term. For the Lack of Fit F-value, it shows a value of 1.31 indicating the Lack of Fit is not significant directly related to the pure error. It is also having the 41.47% chance that a Lack of Fit F-value can be this large related to the noise. This non-significant lack of fit shows a good model to fit.

Table 6: Fit Statistics (CGA concentration)

Std. Dev.	0.0038	**R^2**	0.301
Mean	0.0078	**Adjusted R^2**	0.1612
C.V. %	48.6	**Predicted R^2**	-0.2954
		Adeq Precision	4.1761

Table 6 shows the value of fit statistics from the experimental data. The greater the R-squared in particular, the better the model suits the information. From the fit statistics, the R-squared obtain only has the 0.3010 which means the variability of the response data around its mean is only at 30.1%. However, predicted R-squared from the fit shows a negative value at -0.2954 which implies the overall mean could be a better predictor of the response than the current model. The adequate precision is a measure of the signal relative to the noise ratio. The obtaining value should be greater than 4 which a desirable value for the fit. The ratio shows that value of 4.176 able to indicates an adequate signal. The model equation for the CGA concentration are expressed as shown in equation (2).

$$\text{CGA concentration} = -0.004006 + 0.000048*A + 0.000020*B \quad (2)$$

A: Temperature (°C)
B: Solvent volume (mL)

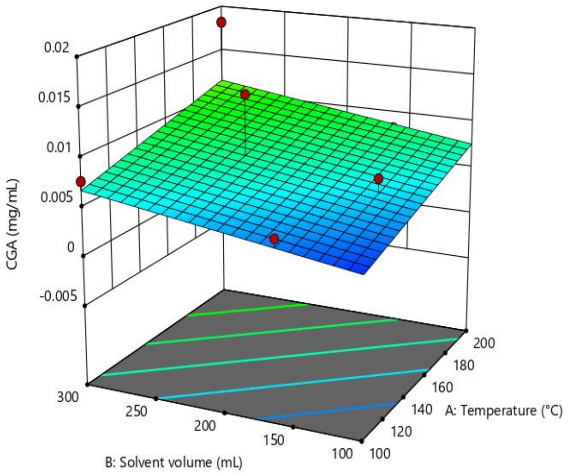

Fig. 3: 3D Response surface plot showing the effect of solvent volume and temperature on the concentration of CGA from GCB extract.

The result of the Soxhlet extraction of GCB that presented in Table 4 are expressed into 3D Response surface plot as shown in Figure 2 where it able to show the trend and predicted condition for the extraction. The yield of CGA for each run of the experiment was determined using equation (1) after UV-Vis analysis. The varying CGA concentration values are indications that the extraction parameters or conditions considerably affect the CGA concentration. It was observed that the yields obtained compared well with the predicted yield by the Design Expert software. The maximum CGA concentration of 0.0182 mg/mL was obtained from the extraction of the corresponding extraction temperature of 200ºC and solvent volume of 300mL.

The maximum value obtained for the yield is likely to be related to the 3D Response surface plot as shown in Figure 2 where the maximum value of predicted CGA concentration only at 0.012mg/mL as shown in Table 7. The 3D Response surface plot obtained shows that higher CGA concentration were optimized at 200°C and 300mL solvent volume. By observing to the 3D Response surface plot, this research was not able to determine optimal conditions for extraction. This because the trend of the predicted value is in increasing manner. In order to determine the optimum condition for extraction, the trend of the 3D Response surface plot should illustrate a downtrend after a certain temperature and solvent volume where the peak of the plot can be the indication of optimum condition for extraction. Nevertheless, the results as shown in Figure 2 indicates higher CGA concentration obtained when temperature and solvent volume increases.

Interaction of extraction temperature with CGA yield from extract has been reported by Azevedo et al. [15] where the extraction temperature has been increased from 50°C to 60°C. This increase in temperature also improves the amount of CGA from GCB extract. However, the research conducted by using supercritical CO_2 extraction aided by co-solvent. Furthermore, increment of temperature does not reaches the value conducted by this research where indicates poor of confirmation of the results. Although the increment of temperature does not range exactly as Soxhlet extraction, the increment of temperature still indicates a significant change of CGA value.

Table 7: Maximum predicted CGA concentration

Number	Temperature	Solvent volume	CGA	Desirability	
1	200	300	0.012	0.59	**Selected**
2	199.598	300	0.012	0.589	
3	198.491	300	0.012	0.586	
4	197.248	300	0.011	0.582	
5	200	289.749	0.011	0.577	
6	200	281.489	0.011	0.567	

According to Sayyar, Abidin, Yunus and Muhammad [16], solvent to solid ratio will affect the extraction as concentration gradient between the solid and the liquid phase becomes greater which favors good mass transfer. It may also be predicted that the variation in the CGA concentration of GCB may be due to the differences in the environmental conditions such as ambient temperature and pressure. Overall, the results indicate that the CGA concentration from the extraction of GCB was affected both of the factor.

B. Antimicrobial analysis with disc diffusion method

After antimicrobial analysis has been conducted on the formulation of hand sanitizer for about 72 hours at 35.8 °C, the zone inhibition on the agar plate was able to be observed. The area that contaminated with E. Coli are in pale yellowish colour as shown in Figure 4. In can be seen that there is zone of inhibition of E. Coli that surrounding the disc enriched with formulated hand sanitizer. This zone inhibition is measured from the centre of the disc to obtain the diameter of zone inhibition. Results of the antimicrobial analysis are presented in Table 8.

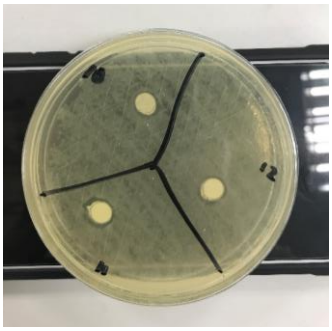

Fig. 4: *E. Coli* Zone inhibition on agar plate

Table 8: Experimental design layout using Central Composite Design for zone inhibition.

Run	Temperature (°C)	Solvent volume (mL)	CGA concentration (mg/mL)	Zone inhibition (mm)
1	100	160	0.0049	10.00
2	200	160	0.0084	12.00
3	100	300	0.0077	11.00
4	200	300	0.0182	13.00
5	79.29	230	0.0021	8.00
6	220.71	230	0.0058	9.00
7	150	131	0.0072	12.00
8	150	329	0.0062	9.00
9	150	230	0.0142	11.00
10	150	230	0.007	9.00
11	150	230	0.0069	11.00
12	150	230	0.0052	10.00
13	150	230	0.0076	10.00

Table 9: ANOVA for Linear model (Zone Inhibition)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	13.36	1	13.36	12.54	0.0046	**significant**
A-CGA	13.36	1	13.36	12.54	0.0046	
Residual	11.72	11	1.07			
Cor Total	25.08	12				

From the ANOVA for linear model as shown in Table 9, it can be seen that the F-value from the model gives out value of 12.54 indicating that the model is significant. The p-value from the ANOVA give out the value of 0.0046 which means that there is a change of the F-value is large occurring due to the nose at about 0.46%. For this linear model terms, it indicates that the model terms are significant since it is less than 0.05.

Table 10: Fit Statistics (Zone Inhibition)

Std. Dev.	1.03	**R^2**	0.5327
Mean	10.38	**Adjusted R^2**	0.4902
C.V. %	9.94	**Predicted R^2**	0.3972
		Adeq Precision	10.1372

From the fit statistics as shown in Table 10, the R-squared obtain only has the 0.5327 which means the variability of the response data around its mean is only at 53.27%. However, predicted R-squared from the fit shows a value at 0.3972 which implies that it is in reasonable agreement with the Adjusted R^2 of 0.4902 and also the difference is less than 0.2. The obtaining value of Adequate Precision should be greater than 4 which a desireable value for the fit. The ratio shows that value of 10.137 able to indicates an adequate signal. The model equation for the zone inhibition are expressed as shown in equation (3).

Zone Inhibition = 8.39629 + 254.91341*A
(3)

A: CGA concentration (mg/mL)

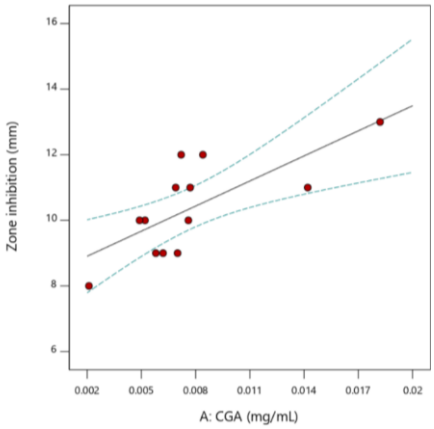

Fig. 5: Effect of CGA concentration toward E.Coli Zone Inhibition

Various yield of CGA concentration has been applied into formulation of hand sanitizer. Hand sanitizer was developed according to the formulation where GCB extract used directly from run/sample 1 to 13. According to the zone of inhibition formed resulting from the developed hand sanitizer with various of CGA concentration against *E. Coli*, different diameter or zone inhibition has been obtained as shown in Table 8. From this antimicrobial analysis, the results of zone inhibition expected to be high when the CGA concentration increases. Research from Lou, Wang, Zhu, Ma and Wang [5] has proven that CGA exhibit antimicrobial properties.

Highest zone inhibition of E.Coli from this experiment is 13mm where the extract has been extracted from solvent volume and temperature at 300mL and 200°C respectively. From Figure 5, the graph predicted from RSM shows an increasing trend of zone inhibition when CGA concentration increases. However, noises of experimental results occur in between CGA concentration at 0.005mg/mL to 0.008mg/mL. The results more likely to be contrast as predicted behavioral of CGA since the extract from lower CGA concentration also able to results in higher zone inhibition.

The expected results should show higher zone inhibition when using GCB extract with higher CGA concentration since antimicrobial properties depends on it. Slightly contradiction of expected results suggested that the experiment has to be conducted with triplicate manner to obtain more accurate results. The effect of zone inhibition still able to prove that green coffee beans extract is able to exhibit antimicrobial properties which have the potential for sanitization application.

IV. CONCLUSION

The maximum conditions predicted for extraction of GCB by using Soxhlet extraction were given as a solvent volume 300mL and extraction temperature of 200ºC with the predicted CGA concentration as 0.012 mg/mL. Based on the findings of this research work (the result of the GCB extraction, CGA concentration, and optimization process), GCB extract has potential as active ingredient for hand sanitizer.

An effective zone inhibition of hand sanitizer was obtained when using GCB extract at 300mL solvent volume and 200°C extraction temperature. The formulation of hand sanitizer resulted in 13mm diameter of inhibition zone which the highest from the experiment. The prediction from Figure 5 also shows that increases in CGA concentration will results in higher zone inhibition. Further analysis of hand sanitizer is suggested in order to investigate the loss or degradation of CGA content with the hand sanitizer formulation.

ACKNOWLEDGMENT

I wish to thank God for giving me the opportunity to embark on my degree and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Dr. Ummi Kalthum Ibrahim. My appreciation goes to the staff and technician of the UiTM who provided the facilities and assistance during sampling. Special thanks to my colleagues and friends for helping me with this project.

References

[1] Lal, M. (2015). Hand Hygiene. International Journal of Current Research, 13448-13449.

[2] Goldberg, J. L. (2017). Guideline Implementation: Hand Hygiene. AORN Journal, 105(2), 203–212.

[3] Yamamoto, T., Tamura, Y., & Yokota, T. (1988). Antiseptic and antibiotic resistance plasmid in Staphylococcus aureus that possesses ability to confer chlorhexidine and acrinol resistance. Antimicrobial Agents and Chemotherapy, 32(6), 932–935.

[4] Dubey, N., Mishra, V., & Thakur, D. (2018). Plant-Based Antimicrobial Formulations. Postharvest Disinfection of Fruits and Vegetables, 211–230.

[5] Lou, Z., Wang, H., Zhu, S., Ma, C., & Wang, Z. (2011). Antibacterial Activity and Mechanism of Action of Chlorogenic Acid. Journal of Food Science, 76(6), M398–M403.

[6] Farah, A., de Paulis, T., Trugo, L. C., & Martin, P. R. (2005). Effect of Roasting on the Formation of Chlorogenic Acid Lactones in Coffee. Journal of Agricultural and Food Chemistry, 53(5), 1505–1513.

[7] Mu, T., Sun, H., Zhang, M., & Wang, C. (2017). Chlorogenic Acids from Sweet Potato. Sweet Potato Processing Technology, 357–403.

[8] Redfern, J., Verran, J., Burdass, D., & Kinninmonth, M. (2014). Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties †. Journal of Microbiology & Biology Education, 15(1), 45–46.

[9] Oliveira, E.R., Da Silva, R.F., Santos, P.R., & Queiroz, F. (2019). Potential of alternative solvents to extract biologically active compounds from green coffee beans and its residue from the oil industry. Food and Bioproducts Processing.

[10] Belay, A., & Gholap, A.V., (2009). Characterization and determination of chlorogenic acids (CGA) in coffee beans by UV-VIS spectroscopy. Afr. J. Pure Appl. Chem. 3 (11), 234–240.

[11] Navarra, G., Moschetti, M., Guarrasi, V., Mangione, M. R., Militello, V., & Leone, M. (2017). Simultaneous Determination of Caffeine and Chlorogenic Acids in Green Coffee by UV/Vis Spectroscopy. Journal of Chemistry, 2017, 1–8.

[12] Balkrishna, A., Ghosh, S., Yadaw, G., Sharma, K., Ghosh, S., & Joshi, S. (2018). Formulation, Evaluation and Antibacterial Efficiency of water-based herbal Hand Sanitizer Gel. Cold Spring Harbor Laboratory.

[13] Ningsih, D. R., Zusfahair, Kartika, D., & Fatoni, A. (2017). Formulation of handsanitizer with antibacterials substance from n-hexane extract of soursop leaves (Annona Muricata Linn). Malaysian Journal of Fundamental and Applied Sciences. 13.

[14] Boyanova, L. (2005). Activity of Bulgarian propolis against 94 Helicobacter pylori strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods. Journal of Medical Microbiology, 54(5), 481–483.

[15] Azevedo, A. B. A.de, Mazzafera, Paulo, Mohamed, R. S., Melo, S. A. B. Vieira de, & Kieckbusch, T. G. (2008). Extraction of caffeine, chlorogenic acids and lipids from green coffee beans using supercritical carbon dioxide and co-solvents. Brazilian Journal of Chemical Engineering, 25(3), 543-552.

[16] Sayyar, S., Abidin, Z. Z., Yunus, R., & Muhammad, A. (2009). Extraction of Oil from Jatropha Seeds-Optimization and Kinetics. American Journal of Applied Sciences, 6(7), 1390–1395.