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Preface

We would like to present, with great pleasure, the inaugural volume-7, Issue-2, February 2021, of a scholarly journal, *International Journal of Engineering Research & Science*. This journal is part of the AD Publications series *in the field of Engineering, Mathematics, Physics, Chemistry and science Research Development*, and is devoted to the gamut of Engineering and Science issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Engineering and Science as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Engineering and Science community, addressing researchers and practitioners in below areas

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Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with IJOER. We are certain that this issue will be followed by many others, reporting new developments in the Engineering and Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOER* readers and will stimulate further research into the vibrant area of Engineering and Science Research.

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Direct analysis by UHPLC-MS/MS of 8 purified fractions from ethanolic extracts of *Taliparitielatum's* flowers in Martinica

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***Abstract*—** *From ethanolic extracts of the flowers of Taliparitielatum (Sw.) eigth different samples were isolated, purified and analyzed by UHPLC-MS/MS to determine the chemical constituents that they content. Each sample was separated and reinjected to get the most possible information about the chemical compounds that they posses. After an exhaustive analysis 8 different chemical components were tentatively identified according to their MS and literature data. Two compounds until remain unknown.*

Keywords*— *Taliparitielatum, ethanolic extract, flowers, UHPLC-MS, chemical components.

I. INTRODUCTION

Taliparitielatum Sw. (Fryxell) of the Malvaceae family is a tree native of Cuba and Jamaica. It is called « Majagua azul » in Cuba, « Mahot bleu » or « Mahot de montagne » in Martinique (US Dpt. of Ag., 2013). It produces beautiful orange to red flowers throughout the year (Figure 1). Domesticated then planted in almost all the islands and countries of the Caribbean basin, it is used for ornamental but also for medicinal purposes. Its flowers are widely used in the Cuban pharmacopoeia for coughs, asthma, catarrh, etc., (Roig, 1974; Acosta y Rodríguez, 2006) where two phytomedication"Imefasma" and"Flormaj" have already been manufactured and marketed in that country.

FIGURE 1: Flowers of *Taliparitielatum* (Sw.) from Cuba and Martinica

Despite widespread use in the Caribbean and undeniable pharmacological potential, there is very little information on its phytochemical constituents in the literature.The ARVARNAM and BIOSPHERES research groups with the University of Havana united in collaborative project therefore undertook to study this plant to characterize its secondary metabolites. The first analyzes by UPLC-DAD-ESI-MS/MS of the flower extract were therefore carried out and published in2017 where the structures of 12 chemical compounds were elucidatedafterisolation. The aim of this research was to analyze eight differentes samples to get information about the chemical components present in each one.

II. MATERIALS AND METHODS

2.1 Plant Material

Flowers were collected in January 2016 along the track road in Balata forest located in Martinica. A voucher specimen is deposited and registered in French Pharmacopeia as Fournet 1752 (4232 Guad). Martinica specimens are registered as *Hibiscus elatus* Sw.

2.2 Solvents

LCMS grade water (Merck), LCMS grade acetonitrile (Merck), LCMS analytical grade ethanol (Merck), and formic acid (Merck) were used in the analysis work. All solvents were degassing previously before used in an ultrasonic bath without filtration.

2.3 Extract and Samples Preparation

Dark red flowering types were collected daily. The isolated petals used were dried in an oven with controlled temperature, at 40°C, during 5 days. The extracts were prepared with the ground material (60 g) without screen extracted in a Soxhlet apparatus with 675 mL of ethanol at 95% during 20 h. The ethanolic extracts were concentrated and evaporated under vacuum to 200 mL at 120 rpm, a temperature of 70 °C and 500 mbar.

For to the purification, 1g of solid was dissolved in 25 mL of diethyl ether and the volume was completed to 100 mL with ethanol. The sample was refrigerated until an abundant solid appear and it was recuperated to filtration. This process was done twice, to obtain only a yellowish-green solid monitoring by TLC on silica gel with fluorescent indicator 254 nm on aluminum cards (layer thickness 0.2 mm) (10 × 20 cm) using n-butanol: acetic acid: water (BAW 4:1:5) as eluent (v/v/v).

2.4 HPLC-DAD-ESI-MS/MS Procedures, Instrumentation, and Parameters

UPLC has been used for the profiling and characterization of the metabolites contained in the extracts. The system used is a Dionex U3000 equipped with a DAD detector having a C18 analytical column (100 x 4.6mm particles 3 µm). Solvent systems: H_2O-0.1% Formic Acid (H_2O) and Acetonitrile-0.1% Formic Acid (Table 1).

TABLE 1
CHROMATOGRAPHIC CONDITIONS (GRADIENT) USED IN THE EXPERIMENTS

Time	Solvents	Gradient
0	H_2O:CH_3CN	80:20
5	H_2O:CH_3CN	80:20
10	H_2O:CH_3CN	0:100
25	H_2O:CH_3CN	0:100
30	H_2O:CH_3CN	80:20
34	H_2O:CH_3CN	80:20

Debit : 0.450 mL/min
UV Detection : 254nm

The HPLC was coupled to a Varian 500MS Mass Spectrometer equipped with an electrospray ionization chamber (ESI) used in negative mode at 5 KV at a capillary temperature of 250 °C. The UV detector and the Mass Spectrometer are used in parallel. A split allows the post column eluent flow to be separated into 2 parts when the flow rate used is greater than 500 µL/min. From a flow rate of 1 mL /min, 400 µL/min are sent to the mass spectrometer and around 600 µL/min to the trash. For the comparison of different powders: Column of 250 mm x ID 4.6 mm, particle 5 µm. The flow rate is 0.800 µL/min. For the product purification: 150 mm x ID 10 mm column, 5 µm particle. Flow rate: 5 mL/min.

Data was acquired in positive or negative mode using the TDDS option "Turbo Data Dependent Scanning" to automatically obtain ion fragmentation spectra that allow the identification of compounds. These mass spectrometry data were compared with free access databases such as “Massbank", "Spider mass DB", the “in-house” database or data from the literature. If this procedure did not allow identification, an attempt to elucidate the structure was carried out manually.

III. RESULTS AND DISCUSSION

Fifty-four peaks were detected here. Thirty temporary identifications, 26 of which were described for the first time in the ethanolic extract of *Taliparitiparitielatum* (Sw.). We mainly found organic acids and flavonoids there. Fragmentation spectra (MS^2) in negative mode were carried out for the structural elucidation of each of the compounds (Figure 2).

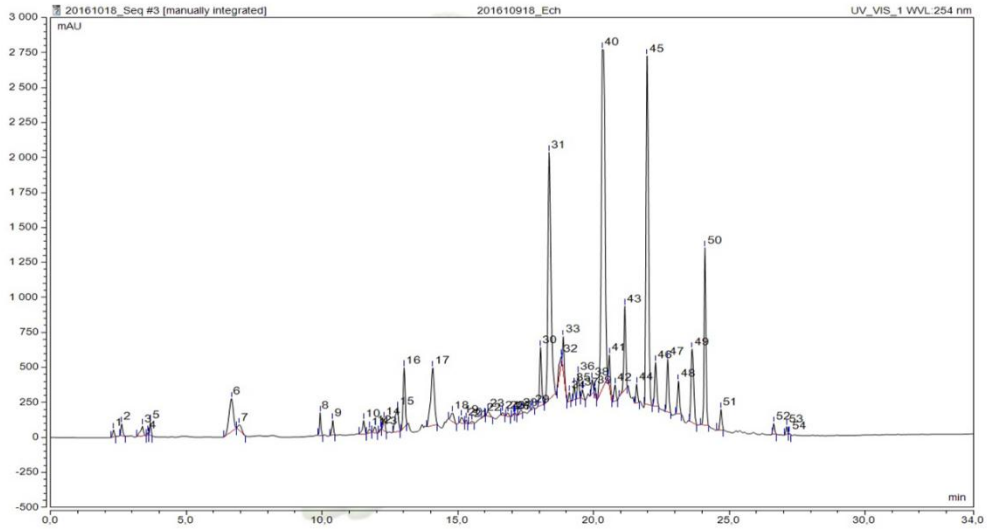

FIGURE 2: UV chromatogram of a hydroalcoholic extract of *Taliparititi elatum* (Sw.) flower recorded at 254 nm. 54 peaks were detected.

We selected the 8 main chromatographic peaks of the flower extracts from *T. elatum* and one by one, they were purified by liquid chromatography. In all cases, each figure corresponds to UV 254nm Chromatograms, as follow: (**A**) RP-UPLC preparative of the Raw sample; (**B**) Analytical RP-UPLC of the raw sample; (**C**) Analytical RP-UPLC of the purified fraction.

The chromatographic peak "F1" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of 2 peaks which correspond to 2 isomers of Dihydrobenzoic acid. The purification of this fraction therefore made it possible to obtain good purification. It is noted that Dihydrobenzoic acid is an intermediate metabolite of degradation of flavonoids according to Bhinu et al., 2002 (Figure 3).

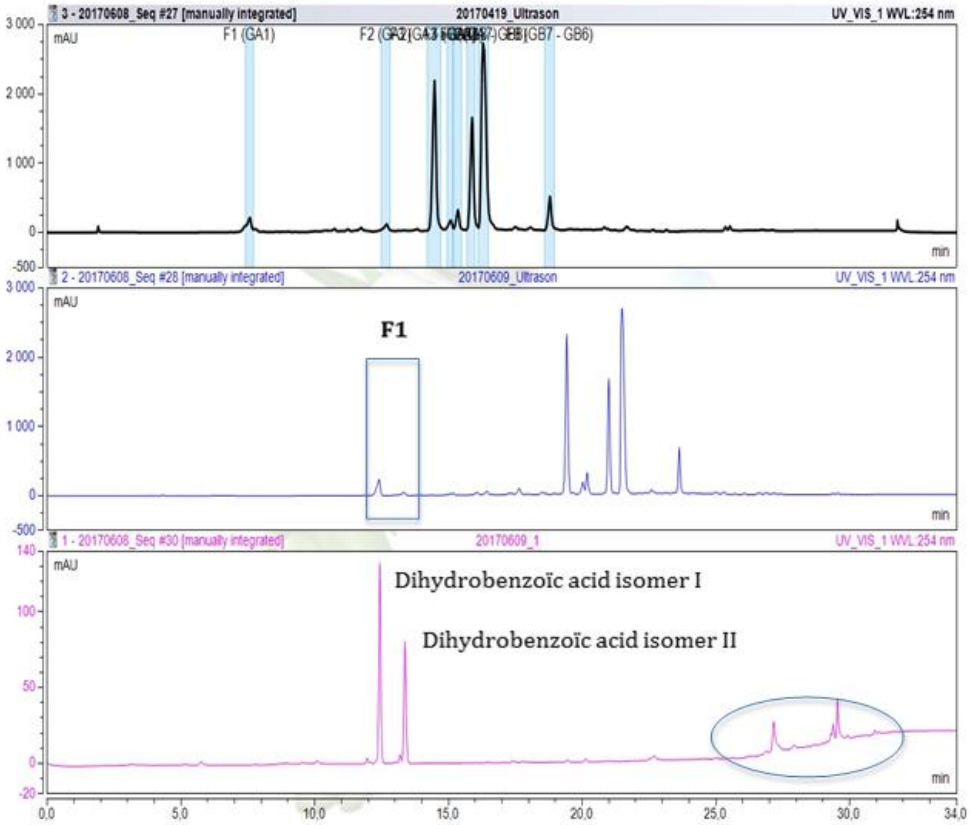

FIGURE 3: Current chromatogram of fraction "F1" isolated and reinjected by UPLC.

The chromatographic peak "F2" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). Very surprisingly, we have no trace of the targeted chromatographic peak during the purification. Several peaks are detected and in particular the peak of the F3 fraction corresponding to the peak of quercetin-*O*-sambubioside. We also observe the presence of the isomers of dihydrobenzoic acid. Contamination by the F3 fraction and post-harvest degradation are possible (Figure 4).

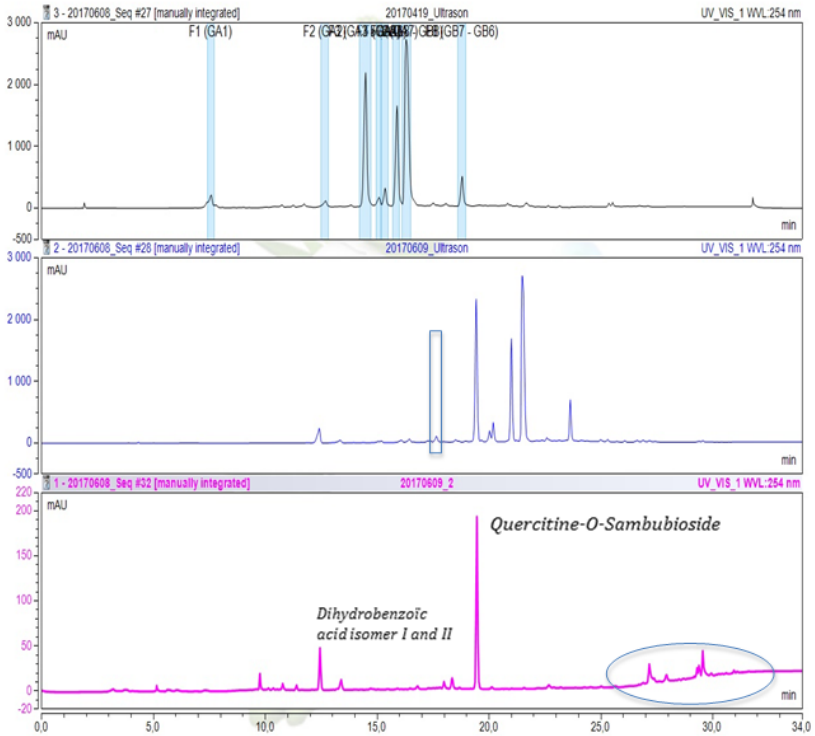

FIGURE 4: Current chromatogram of fraction "F2" isolated and reinjected by UPLC

The chromatographic peak "F3" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of a single majority peak which corresponds to Quercetin-*O*-sambubioside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 5).

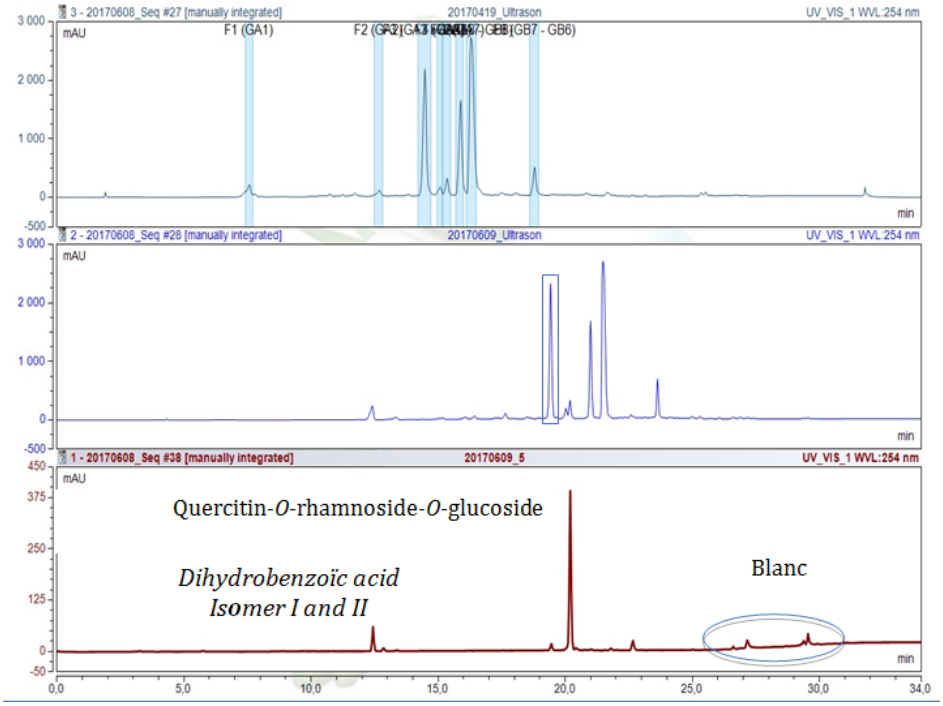

FIGURE 5. Current chromatogram of fraction "F3" isolated and reinjected by UPLC.

The chromatographic peak "F4" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). Very surprisingly, the targeted chromatographic peak during the purification is present, but it is far from being the majority peak. Several peaks are detected and in particular the peak of the F4 fraction corresponding to the peak of quercetin-*O*-rhamnoside-*O*-glucoside.Analysis of mass spectrometry data did not allow the identification of the product that was detected before the biggest peak near the 20 min of retention time.Contamination by the F5 fraction and post-harvest degradation are possible (Figure 6).

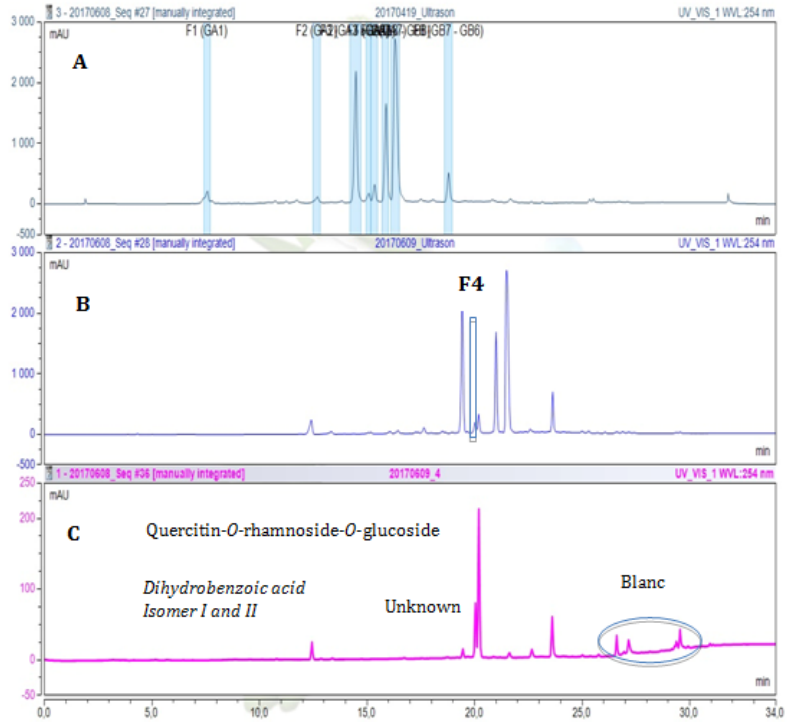

FIGURE 6: Current chromatogram of fraction "F4" isolated and reinjected by UPLC

The chromatographic peak "F5" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of a single majority peak which corresponds to Quercitin-*O*-rhamnoside-*O*-glucoside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 7).

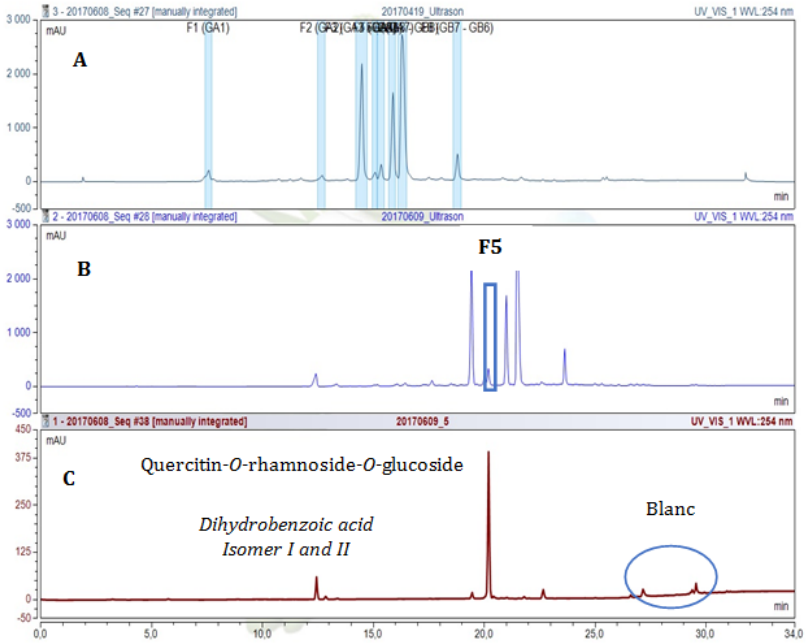

FIGURE 7: Current chromatogram of fraction "F5" isolated and reinjected by UPLC

The chromatographic peak "F6" was collected by RP-UPLC (Fig. A). This fraction was harvested and reinjected (Fig. C). We note the presence of a single major peak which corresponds to Quercitin-*O*-glucoside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 8).

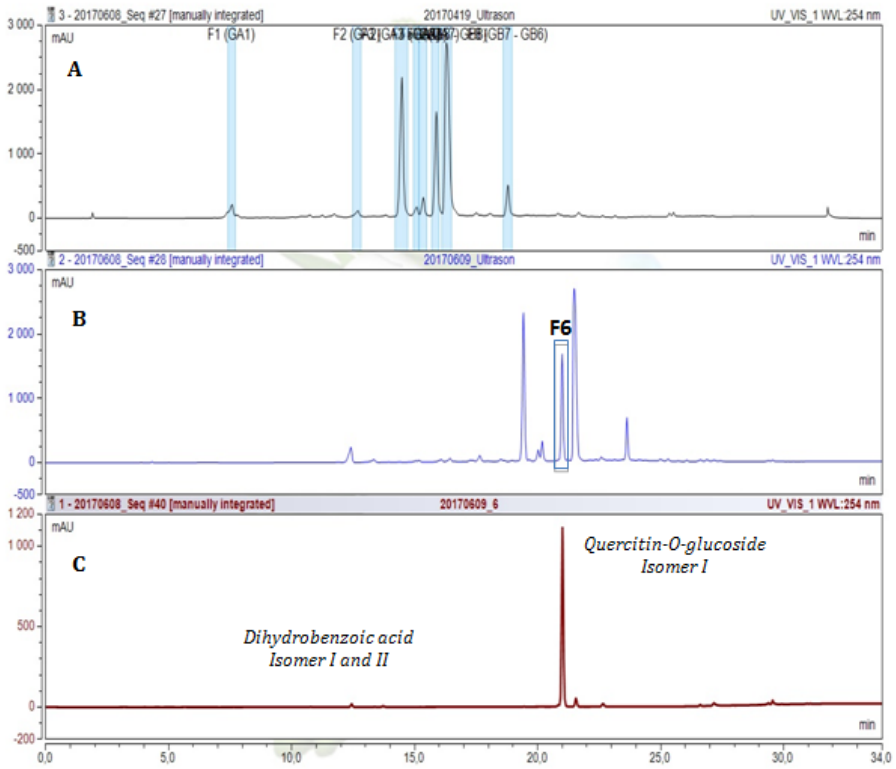

FIGURE 8: Current chromatogram of fraction "F6" isolated and reinjected by UPLC

The chromatographic peak "F7" was collected (Fig. A) by RP-UPLC. This fraction was harvested and reinjected (Fig. C). We note the presence of a single majority peak which corresponds to Gossypetin-*O*-glucoside (Mass spectrometry data not shown here). As we would expect, we mostly got the targeted chromatographic peak during the purification (Figure 9)

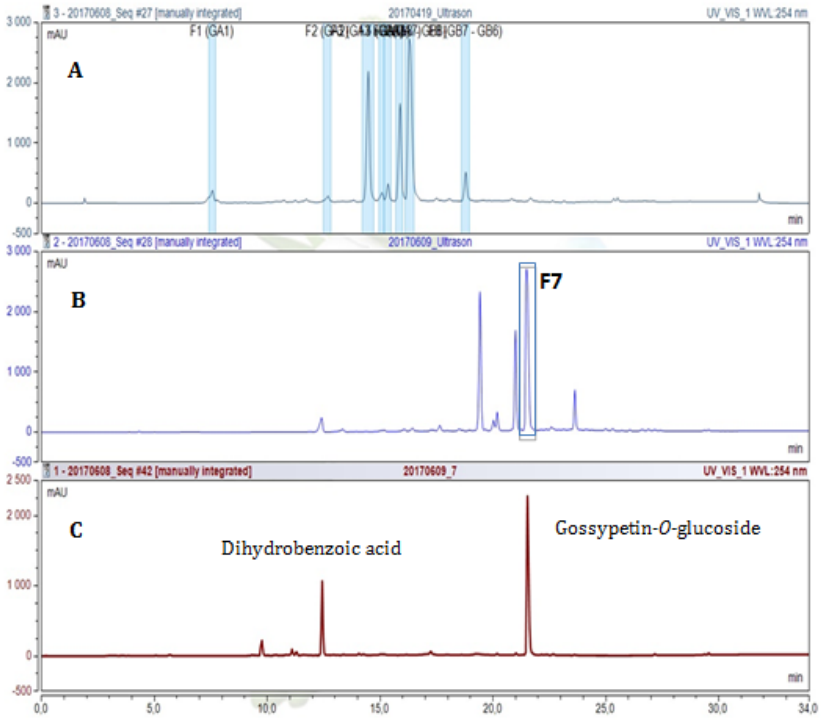

FIGURE 9: Current chromatogram of fraction "F7" isolated and reinjected by UPLC

The chromatographic peak "F8" was collected (Fig. 10 A) by RP-UPLC. This fraction was harvested and reinjected (Fig. 10 C). We note the presence of three majority peaks: Quercitin-*O*-glucoside, dihydrobenzoic acid and dihydroxyphenyl propionic acid (Mass spectrometry data not shown here). A surprising result knowing that we could expect a single majority peak. The most likely hypothesis here is post-harvest degradation since dihydroxybenzoic acid and dihydroxyphenyl propionic acid are intermediates in the degradation of Flavonoids (Bhinu et al., 2002).

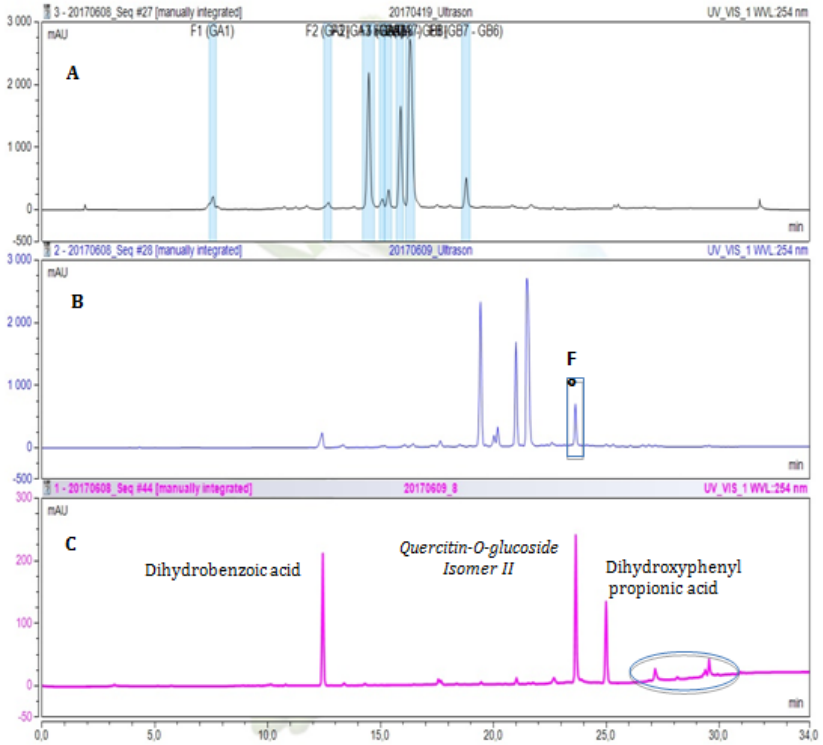

FIGURE 10: Current chromatogram of fraction "F8" isolated and reinjected by UPLC

Summarizing, the presence of three different kinds of organic acids were detected in ethanolic extracts of the petals from the flowers of *T. elatum* (Sw.) in Martinica, taking into account that dihydrobenzoic acid was detected presumably in two isomeric forms from fraction 1 to 6, while in fractions 7 and 8 this chemical component was found as a single peak. Another one was the dihydroxyphenyl propionic acid. Five different kinds of flavonoids were detected in the fractions, such as, quercitin-*O*-sambubioside, quercetin-*O*-rhamnoside-*O*-glucoside, quercetin-*O*-glucoside (in two isomeric forms), and gossypetin-O-glucoside.

Until now, we have not evidence of the presence of those chemical components in the petals of the flower of *T. elatum* (Sw.) in Martinica, for that reason our research team is proposing for the first time the mentioned chemical compounds into ethanolic extracts of this flower using UPLC-MS/MS.

Is notably that perhaps we are in the presence of two new flavonoids as phytochemical components of the petals in this specie that belongs to gossypetin derivatives: gossypetin-8-O-glucoside or gossypin, previously reported in the flowers of *Hibiscus sabdariffa* (Visweswara and Seshadri, 1946) and *Hibiscus vitifolius* (Subramanian and Nair, 1972) and gossypetin-3-*O*-glucoside or gossytrin, reported in the flowers of *Hibiscus sabdariffa* (Seshadri and Thakur, 1961) and *Hibiscus tiliaceum* (Nair et al., 1961). Both chemical compounds have the same chemical formula ($C_{21}H_{20}O_{13}$) and the same molecular mass (480u). They differ only in the position of the glucose moiety.

Quercetin-*O*-glucoside, quercetin-*O*-sambubioside, and quercetin-*O*-rhamnoside-*O*-glucoside were reported for the first time by our research team in 2017, but in the extracts of the flowers of *T. elatum* in Cuba (González et al., 2017). Those chemical compounds were previously reported in the petals and calyces of *H. sabdariffa* (Inmaculada et al., 2009; Beltran-Debon et al., 2010).

Both isomers of quercetin-*O*-glucoside could be attributed to isoquercitrin (quercetin-3-*O*-glucose) and hiperoside (quercetin-3-O-galactose) according to its molecular masses (464u) previously reported in the petals of the flowers of *T.*

elatum in Cuba by our research team in 2016 (Yaque, J.G. et al., 2016) and reported in two *Peumo* trees in Chile (Simirgiotis, 2013).

IV. CONCLUSIONS

A simple and versatile analytical method, the "UPLC-DAD-ESI-MS / MS" was implemented to allow direct identification of the constituents inhydroalcoholic extract of the flower petals of *Taliparitielatum* (Sw.). The analysis was carried out by RP HPLC coupled to a DAD detector and to a tandem ion trap mass spectrometer in order to obtain a UV profile and a spectrum of fragmentations in negative mode making it possible to achieve provisional identification. This study was also accompanied by the analysis of the extraction precipitate and the first attempts to purify the majority constituents of the extract. Eights compounds have thus been tentatively identified, all of them reported for the first time in the ethanolic extracts of this part of the plant. Thus, it has been demonstrated that gossypetin-*O*-glucoside does not precipitate alone and that the use of UPLC-DAD for preparatory purposes is promising. We were able to isolate four major products including gossypetin-*O*-glucoside.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] U.S. Department of Agriculture. *Hibiscus elatus* Sw. "mahoe".Natural ResourcesConservationService. PlantsDatabase.2013.

[2] Roig, J. T. Plantas Medicinales Aromáticas y Venenosas de Cuba. Editorial Científico Técnica, La Habana, 618-619, 1974.

[3] Acosta de la Luz, L. L.; Rodríguez Ferradá, C. A. Plantas medicinales. Bases para una producción sostenible. Agrinfor, Impresiones MINAG, 124-125, 2006.

[4] Bhinu V. S. Pillai and SanjaySwarup. Elucidation of the Flavonoid Catabolism Pathway in *Pseudomonas putida* PML2 by Comparative Metabolic Profiling.Applied and Environmental Microbiology.Vol. 68,p. 143–151, 2002.

[5] VisweswaraRao K, Seshadri TR. Colouring matter of the flowers of *Hibiscus vitifolius*. Proc. Indian. Acad. Sci. (Math. Sci.).1946; 24:352-356.

[6] Subramanian SS, Nair AGR. Flavonoids of four Malvaceous plants.Phytochemistry. 1972; 11(4):1518-1519.

[7] Seshadri TR, Thakur RS. Constitution of gossytrin, a new glycoside from the flower petals of *Hibiscus sabdariffa*.Journal of the Indian Chemical Society.1961; 38:649-665.

[8] Nair AGR, Subramanian SS, Swamy MN.Glycosides from the flowers of *Hibiscus tiliaceus*.Journal Scientific Industrial Research (India). 1961; 20B:553.

[9] González J, Cuéllar A, Sylvius L, Verdeau F, François-Haugrin F, Smith-Ravin J, Marcelin O. New Phytochemical Profile of Ethanolic Extract from *Taliparitielatum* (Sw.). Journal of Pharmacy and Pharmacology 5 (2017) 489-496. doi: 10.17265/2328-2150/2017.08.001

[10] Inmaculada C. Rodriguez et al. Medina Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap MS J. Sep. Sci. 2009, 32, 3441–3448.

[11] R. Beltran-Debon et al., The aqueous extract of *Hibiscus sabdariffa* calices modulates the production of monocyte chemoattractant protein-1 in humans. Phytomedicine 17 (2010) 186–191.

[12] Yaque, J.G., Cuéllar, A., Massi, L., Monan, M., Nossin, E. and François-Haugrin, F. (2016) Isolation and Characterization of Flavonols by HPLC-UV-ESI-MS/MS from *Taliparitielatum*S.w. American Journal of Plant Sciences, **7**, 1198-1204. http://dx.doi.org/10.4236/ajps.2016.78115

[13] Simirgiotis, M.J. Antioxidant Capacity and HPLC-DAD-MS Profiling of Chilean Peumo (*Cryptocarya alba*) Fruits and Comparison with German Peumo (*Crataegusmonogyna*) from Southern Chile. Molecules.2013; 18: 2061-2080.

Performance Evaluation of Bentonite Muds Formulated using Cassava Starch Treated with Preservatives

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Abstract— *Extensive works have been done on the subject matter of local sourcing of drilling fluid additives to reduce our importation burdens especially in this era of dwindling oil fortunes. This local sourcing will bolster industrialization and reduce unemployment in a vast economy like Nigeria. The results of the previous studies have shown promising potentials which are in tandem with our local content mandate of the government. Cassava starch has been identified as one of these additives and our ranking as the world largest producer of cassava remains instructive. Currently, all the starch used in the oil and gas industry is imported. The major constraint to the use of cassava starch is their extreme susceptibility to post-harvest degradation. This work, therefore, is an attempt to address this problem of stability by the application of appropriate preservatives without prejudice to the rheological properties of the mud. In carrying out this study at temperatures of 80, 120, 150 and 190^{0}F, three cassava starch cultivars TMS 92/0057, TMS 98/0581 and TMS 96/1632 and four common preservatives in the food industry; the salts of benzoate, propionate, sorbate and metabisulphite were used in the bentonite mud formulations. The result showed that out of the sixty-one mud formulations, only five of them adequately met the API rheological properties threshold. These five muds exhibited properties that compare favourably to the imported starch sample. This stabilized product holds much promise as a substitute to the imported starch for use in water-based drilling mud formulations for the Nigerian oil industry.*

Keywords— Cassava starch, Drilling fluids, Rheology, Preservatives.

I. INTRODUCTION

The use of preservatives predates history. The prehistoric men were known to have preserved their perishables with substances such as salt and vinegar [1]. The ante of the preservation industry has since then moved up to the present day whereby it is almost possible to preserve anything so desired. More often than not, this is done through a myriad of processes and operations that sometimes see a combination of such, to achieve some complex and even conflicting requirements [2].

A preservative is a substance that is added to a product to maintain an existing condition or prevent decomposition by microbial attacks or degradation through other undesirable changes. However concerning food preservation which starch belongs to, preserving what is, may not be just adequate as additional requirements of improving flavour, texture and visual appearance may also be imposed [3].

Preservatives are classified into two groups according to the form of attack as antimicrobial or antioxidants. The anti-microbial preservatives prevent the attacks from micro-organisms such as moulds, yeasts and bacteria and some common ones are the salts of benzoate, sorbate, propionate and metabisulphite [4]. The antioxidants prevent the oxidation of foods and especially those containing unsaturated fats and oils. It is this oxidation which produces the rancid taste in such foods and typical examples are the butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and the ascorbates [5].

Cassava (*Manihotesculenta*) is a major root crop largely grown in the tropics and subtropical regions of the world and over a wide range of soil and environmental conditions. It is because of its commonness that it is sometimes described as the "Bread of the Tropics" [6]. Documented reports of well over 40 cassava varieties have been identified and Nigeria is the world's largest producer [7]. According to [8], the estimated world production in 2017 was 278 million tonnes, out of which Nigeria was accountable for 20%. Cassava demonstrates an amazingly stubborn resilience in that it can survive in the least of

farmland and environmental conditions [9].Cassava is a reliable and convenient source of food for millions of Nigerians and it is estimated that more than 90% of our cassava production is processed into food [10]. However, the use of cassava and cassava products amongst which is starch has been finding wider applications other than the normal food industry over the years, such as biofuels, animal feeds, laundry, beverages and drilling fluids

Starch is employed in the oil and gas drilling fluid formulations as secondary viscosifiers and filtrate reducers to improve rheological properties and filtration characteristics, respectively [11], [12]. Starch is a polysaccharide consisting of the crystalline amylose and the amorphous amylopectin, with the latter accounting for the gelling properties [13]. As a polymeric material, it exhibits thixotrophy and it is this property that has informed its application in the formulation of drilling fluids [Thomas, 1982 as cited by 14].Additives that address specific requirements are also incorporated into the muds to function satisfactorily under varied down-hole conditions. According to [15], present-day muds are, therefore, formulated to address these key functions; controlling formation pressures, cooling and lubricating the drilling string/bit, removing of cuttings from the borehole, maintaining wellbore stability, transmitting hydraulic energy to the bit, suspension of cuttings in the borehole, protecting permeable zones by building a filter cake, supporting of the drill string, releasing of the cuttings at the surface, corrosion prevention, data logging and reducing filtration rate.

The major drawback to the use of cassava and cassava products is their ready susceptibility to post-harvest physiological degradation, infestations and inherent deterioration as a result of micro-organism activities [16]. This has not only brought severe losses to the farmers but has more importantly limited their potential for usage by our oil and gas industry and by extension, the export market. [Farquet and Fargette, 1990 as cited by16], has reported losses of 20-90 % in 15 African countries, Nigeria inclusive.

Previous works on the use of local cassava starches for drilling fluid applications for exploration and exploitation activities in the petroleum industry have demonstrated good and promising potential as compared to the imported ones [13],[14],[17]. However, the equally important study on how these starches can be stabilized for storage with acceptable shelf life, have not been fully addressed. The over $20 billion annual spends in the Upstream oil and gas industry have largely benefited the import market much to our detriment and gravely against the Nigerian government mandate through the Nigerian Content Development and Management Board (NCDMB), that 60% threshold of the chemicals/additives in the industry be sourced locally [18]. Therefore, advancing the course of knowledge to the ultimate market square economically is predicated on effective closure of this stability gap. The objective of this work, therefore, is to investigate and evaluate the performance of starches that were treated with preservatives for water-based drilling fluid formulations.

II. MATERIALS AND METHODS

Three 12-months old cassava cultivars TMS/92/0057, TMS98/0581 and TMS96/1632 were obtained and processed at the National Root Crop Research Institute (NRCRI), Umudike, Umuahia, Nigeria. Imported starch as a control sample (Amidon), bentonite and four common preservatives in the food industry were obtained from an industrial chemical supply company in Port Harcourt. These preservatives were the salts of benzoates, sorbates, propionates and sulphites.

Equipment used includes: mechanical grater, thermos- regulated oven tray drier, viscometer (OFITE model 800), API filter press (OFITE model), electronic balance, Hamilton Beach mixer, muslin cloth, measuring cylinder, beaker, spatula, 150-micron sieving mesh, stopwatch and mud balance.

2.1 Extraction of the starch

Each of the three cassava cultivars was subjected to starch extraction process as described by [14], [19]. Freshly harvested tubers were washed with potable water, peeled with a stainless knife and again washed thoroughly to remove all dirt and sand. The tubers were ground and sieved with the addition of a small quantity of portable water to facilitate the sieving operation. The filtrate was allowed to settle for 4 hours and then decanted. This left a white, tasteless and odourless starch at the bottom of the container. The wet starch was spread thinly over an aluminium tray for open-air drying at atmospheric conditions of 27^0C-30^0C for about 6hours to minimize damage to native starch granules. The starch was further dried in an air oven for about 6 hours at 60^0C. The dried starch was finally milled in blenders to fine particles and sieved with 150-micron mesh.

2.2 Mud Preparation

Following [21] specification, and as shown in Table 2.1, bentonite mud was individually formulated with the three cassava starch cultivars TMS92/0057, TMS98/0581, TMS96/1632 and the imported starch sample (Amidon) with distilled water as

the continuous phase. The local starches were earlier treated with the four preservatives, at five different concentrations before the mud formulation exercise. Muds A1 to A15, B1 to B15, C1 to C15 and D1 to D15 were treated with the benzoate, propionate, sorbate and metabisulphite salts, respectively. The preservatives were the commonly encountered ones in the agricultural industry [21].

TABLE 2.1
BASIC MUD RECIPE

Mud Type	1% Starch(TMS92/0057)	Bentonite	Distilled Water	% Benzoate salt preservative
A1	0.23g	22.5g	350ml	0
A2	0.23g	22.5g	350ml	0.05
A3	0.23g	22.5g	350ml	0.1
A4	0.23g	22.5g	350ml	0.5
A5	0.23g	22.5g	350ml	1.0

2.3 Rheological Properties Determination

The Bentonite-starch muds were variously formulated as detailed. Each of the mud suspension was poured into a viscometer cup and subjected to a multi-speed Fann Viscometer which was used to carry out the viscosity test at the shear speeds of 600, 300, 200, 100, 60, 30, 6 and 3 rpm, respectively. The viscosity tests were carried out at temperatures of 80°F, 120°F, 150°F, and 190°F respectively. The control sample (Amidon) mud was formulated by adding 1% of the starch to the bentonite and distilled water followed by thorough mixing. The only difference was that no preservative was added to the suspension. This was again subjected to a viscosity test at the same various temperatures both before and after ageing. The results were as shown in the graphs. The results from the viscometer were used to calculate the rheological properties by the following equations [22].

Plastic viscosity (cP) = 600rpm reading–300rpm reading (1)

Yield Point = 300rpm reading – Plastic velocity (cP) (2)

Apparent Viscosity (cP) = 600rpm reading /2 (3)

Yield stress = (2x3rpm reading) – 6rpm reading (4)

Shear rate (sec^{-1}) = rpm x 1.703 (5)

Shear stress (Pa) = Dial reading x 1.065 (6)

III. RESULTS AND DISCUSSION

The rheological characterization of the muds are presented and discussed with the [23] as the basis.

3.1 Mud Viscosities

The viscosity performances for these muds before ageing were presented in Figures 1 - 3 for formulations with starches from TMS 92/0057, TMS 98/0581 and TMS 96/1632 cassava cultivars respectively. The performance of the control sample (Amidon) was also included for ease of better comparison.

All the muds from TMS92/0057 met the API minimum viscosity of 30 cP at the speed of 600 rpm and temperature of 80^{o}F as the muds posted a viscosity range of 30-58 cP (Figure 1). Generally, muds containing the sulphite salt in its formulation showed the highest viscosity at a concentration of 1.0 per cent (D5). This was followed by muds with sorbate, propionate and benzoate, in that order.

Similarly, all the TMS 98/0581 muds met the threshold. Metabisulphite preserved starch muds again indicated the highest viscosity of 56 cP (D10), with increasing concentrations, followed by muds with propionate, sorbate and benzoate also in that order. All the muds met the API minimum viscosity threshold and ranged from 32 to 57cP. At the highest concentration of 1.0 per cent concentration, sulphite containing muds indicated the highest viscosity of 57cP, followed by benzoate, propionate and lastly the sorbate.

Figure 3 shows the profile of muds from TMS 96/1632. All the muds met the API minimum viscosity threshold and ranged from 32 to 57cP. At the highest concentration of 1.0 per cent concentration, the metabisulphite containing muds indicated the highest viscosity of 57cP, followed by benzoate, propionate and lastly the sorbate.

All mud formulations met the minimum threshold in terms of mud viscosity only and this is in tandem with the works of some researchers that our local cassava starches had promising viability for application in water-based drilling formulations as a viscosifying agent [13], [14], [17], [24].

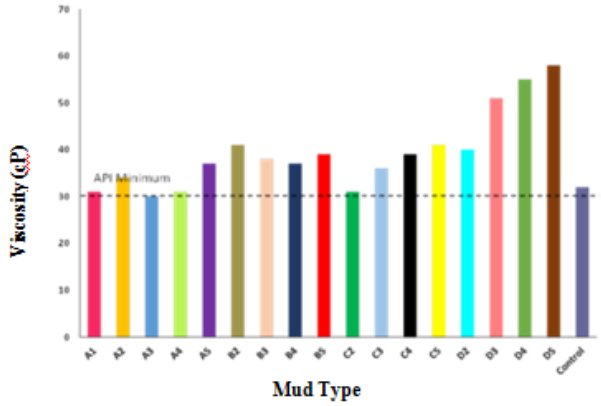

FIGURE 1: Mud Viscosity for TMS92/0057 starch muds treated with different preservative

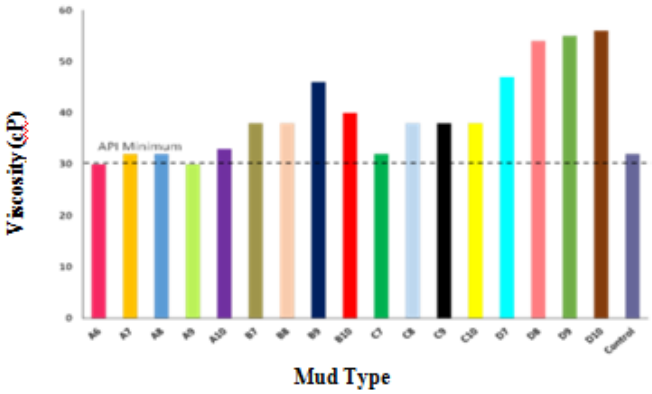

FIGURE 2: Mud Viscosity for TMS98/0581 starch muds treated with different preservatives

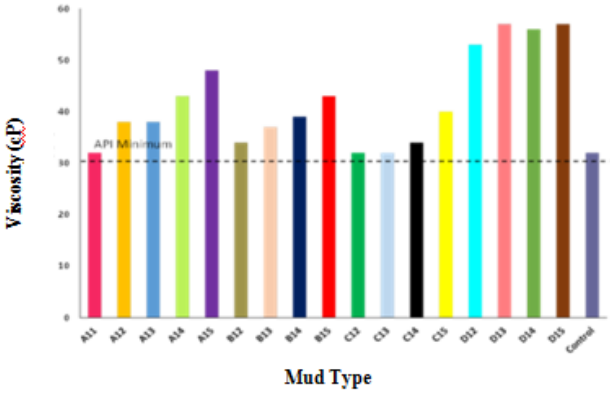

FIGURE 3: Mud Viscosity for TMS96/1632 starch muds treated with different

3.2 Yield Point of Mud Formulations

The presentation of yield point values of the three cassava starch cultivars as formulated are shown in Figure 4-6. All the muds from TMS 92/0057 cultivar were within the threshold of 15-45lb/100ft^2 [23] except for muds D3, D4 and D5. Comparable results were reported by [24]. The TMS 98/0581 muds all met the threshold whereas only mud D15 of the muds from TMS 96/1632 formulations failed the specification target with a value of 51lb/100ft^2.The mud yield point is a measure of its cuttings carrying capacity. Higher values have negative impacts on the mud pump, wellbore stability, borehole cleaning, amongst others due to pressure considerations [26].

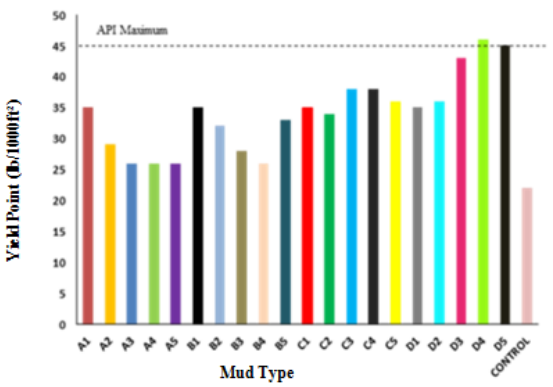

FIGURE 4: Yield Point for TMS92/0057 starch-bentonite formulated muds

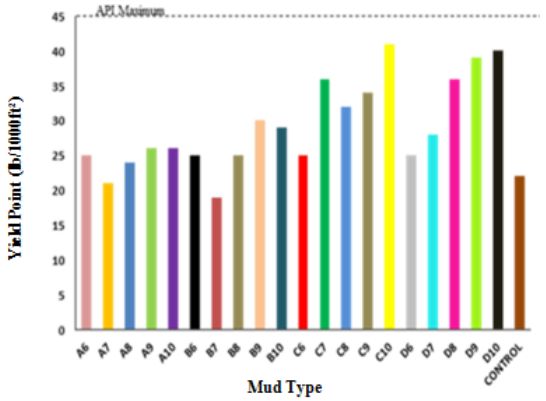

FIGURE 5: Yield Point for TMS98/0581 starch-bentonite formulated muds

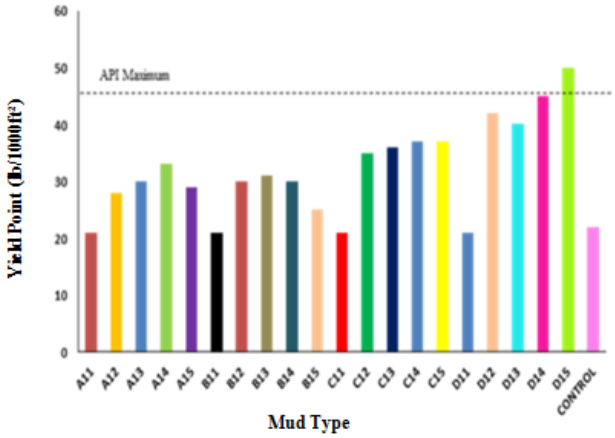

FIGURE 6: Yield Point for TMS96/1632 starch-bentonite formulated muds

3.3 10-Seconds Gel Strength of Mud Formulation.

The 10-second gel strength values of muds formulated with the three starch cultivars TMS 92/0057, TMS 98/0581 and TMS 96/1632 were presented as in Figures 7, 8 and 9 respectively. Four of the muds from TMS 92/0057 formulations (A2, A3, A4, C2) and TMS 96/1632 formulations (A12, B12, C12, C13) have gel strength values within the API Specification of 3-20lb/100ft^2 [23], while for the TMS 98/0581 formulations, eight muds (A6, A7, A8, A9, B6, C6, C7, D6) met the threshold. Reference [24] had reported comparable results for cassava starch bentonite muds. Mud gel strength is an indication of the cuttings suspension capacity of the mud under static conditions. Managing operational drilling challenges such as cessation of fluid flow, caused by pump failures could lead to catastrophic damages but for a good controlled gel strength value. The interaction between the mud components and the preservatives at their various concentrations may be responsible for these observations.

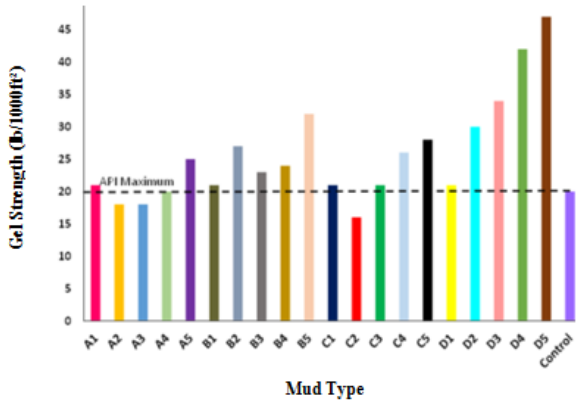

FIGURE 7: 10 Secs Gel Strength for TMS92/0057 Starch-Bentonite formulated muds

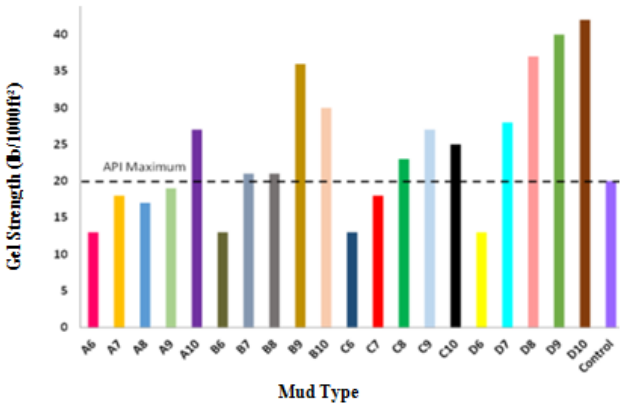

FIGURE 8: 10 Secs Gel Strength for TMS 98/0581 Starch-Bentonite formulated muds

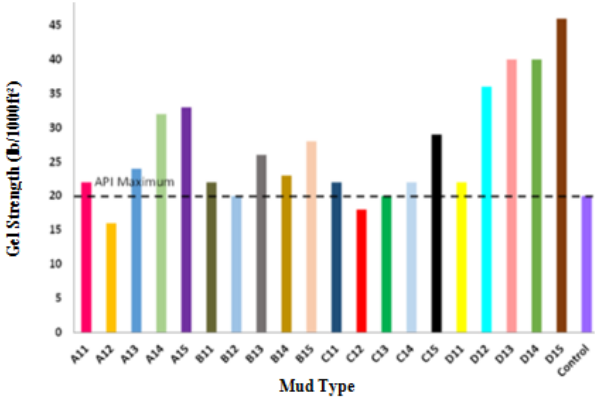

FIGURE 9: 10 Secs Gel Strength for TMS 96/1632 Starch-Bentonite formulated muds

3.4 10-Minutes Gel Strength of Mud Formulation.

The 10- minutes gel strength values of muds formulated with the three starch cultivars were presented as in Figures 10to 12. These six muds; B2, B5, D2,D3, D4, D5, failed to meet the API specification threshold of 8-30lb/100ft^2 for the TMS 92/0057, seven muds (A10, B9, B10, D7, D8, D9, D10, for TMS 98/0581 formulations and eight muds (A14, A15, B15, C15, D12, D13, D14, D15) of TMS 96/1632 formulations were also above the threshold. Mud gel strength is an indication of the cuttings suspension capacity of the mud under static conditions. According to [27], flat rheology phenomenon is critical for effective drilling operations and this implies a small differential between the 10-seconds and the 10-minute gel strength values as indicated by most of these formulations.

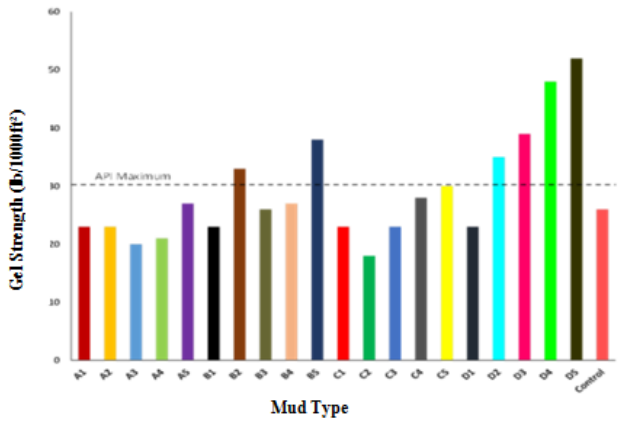

FIGURE 10: 10 Min. Gel Strength Profiles for TMS 92/0057 Starch-Bentonite formulated muds

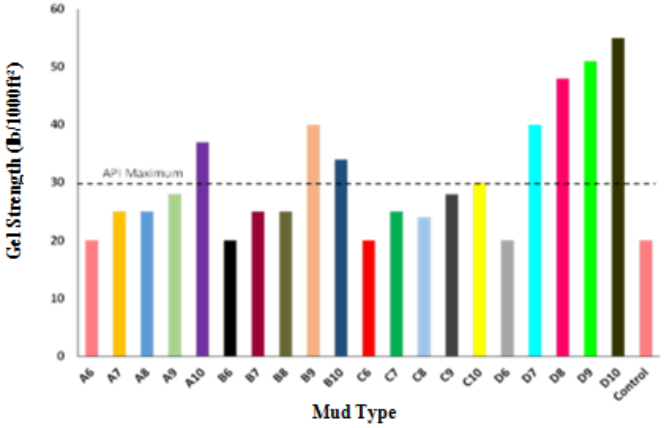

FIGURE 11: 10 Min. Gel Strength Profiles for TMS 98/0581 Starch-Bentonite formulated muds

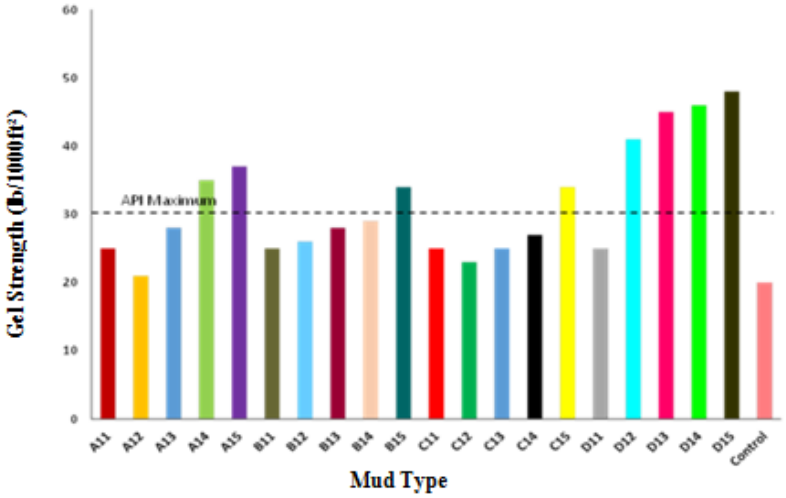

FIGURE 12: 10 Min. Gel Strength Profiles for TMS 96/1632 Starch-Bentonite formulated muds

3.5 Muds that met AP1 Specifications

A determination and analyses of the rheological characterization of all the mud samples from the three cassava starch cultivars of TMS 92/0057, TMS 98/0581 and TMS 96/1632 treated with the four preservatives at varying concentrations were made. The analyses were made concerning the AP1 standard specifications (AP1 2004). It was only the following five muds that met the threshold of the specifications; A2, A7, A8, B7 and C7.

Muds A7, C7, A8 and B7 were all formulated with TMS 98/0581 starch cultivar, containing 0.05% benzoate, 0.05% sorbate, 1% benzoate and 0.05% propionate, respectively. Mud A2 was a formulation of TMS 92/0057 with 0.05%benzoate. In advancing this work, therefore, it is evident that the benzoate could be considered as an appropriate preservative at low concentrations in formulation with TMS 98/0581 and TMS 92/0057 starch cultivars. It is instructive to note that the findings of previous researchers on this subject matter posited similar observations as to the promising potentials of these cassava starch cultivars as effective colloids for water-based drilling fluids formulations][13, [17]. Most importantly, the

characterization of the control starch mud sample E1 (Amidon) compares very favourably with these recommended samples but particularly mud samples A2 and A7.

IV. CONCLUSIONS

In this work, a total of sixty-one mud samples were prepared and analyzed. The deliberate and elaborate sampling and analyses were to ensure that the results will make a good contribution towards the closure of the work that has been done towards the industrial application of local cassava starches in drilling fluid formulations. Accordingly, we conclude as follows:

The application of benzoate preservatives at low concentration of 0.05% to each of the two cassava starch cultivars holds much promise as a substitute to the imported starch, for use in water-based drilling mud formulations for the Nigerian oil and gas industry

Both mud formulations showed similar rheological characterization with the imported starch sample (control sample) and all the parameters conformed to the API standard.

The last step in these positive closure efforts is ongoing and that will be the deployment of moisture sorption studies to validate the acceptable shelf life for these stabilized cassava starch products.

REFERENCES

[1] G. Evans, B. de Challemaison, and D.N. Cox, "Consumers' ratings of the natural and unnatural qualities of foods".*Appetite,*vol.54, no.3, pp. 557–563June 2010.

[2] E. Gayán, J.A. Torres, andD. Paredes-Sabja, "Hurdle Approach to Increase the Microbial Inactivation by High-Pressure Processing: Effect of Essential Oils",*Food Eng. Review*,vol.4, pp. 141-148, 2012.

[3] I. Leistner, "Basic Aspects of Food Preservation by Hurdle Technology," *International J. Food Microbiology,* vol. 55 pp.181–186, 2000.

[4] L. Dalton, "Food Preservatives," *Chemical and Engineering News*, vol. 80 no 45, pp. 40, November 2002.

[5] T.A.M. Msagati, "The Chemistry of Food Additives and Preservation," Oxford, London, U.K., Wiley-Blackwell Publishers, pp. 1-25, 2012.

[6] C. Adams, R. Marieta,A. Siqueira, W. Neves, and R. Sanches, " Bread of the Land*:* The Invisibility of Manoic in the Amazon. Amazon Peasant Societies in a changing Environment," New York, U.S.A. Springer Publishing, pp. 281-305, 2009.

[7] FAOSTAT, "Food and Agricultural Organization Statistics: Production, Crops, Cassava," Food and Agricultural Organization, 2014.

[8] C. Chuasuwan, "Cassava Industry, Thailand Industry Outlook 2018-2020,"Krungsri Research, 2018 pp.1-8.

[9] G.D. Stone, "Both Sides Now," *Current Anthropology*, vol. 43, no 4, pp. 611- 630, 2002.

[10] F.I. Nweke, and V. Manyong, "Adoption of Improved Cassava varieties in Nigeria," COSCA Working Paper 20,pp. 6-19, 2000.

[11] O.A. Falode, O.A. Ehinola, and P.C. Nebeife, "Evaluation of local bentonitic clay as oil well drilling fluids in Nigeria," *Application of Clay Science*, vol.39, pp. 19-27, 2008.

[12] A. Bentriou, K. Fourar, A. Benmounah, and B. Safi, "Comparative study of biopolymers action on physico-chemical and rheological properties of water-based drilling muds," *Asian J.Chemistry,* vol. 26 no 14, pp. 4386–4390, 2014.

[13] T.F.Harry, O.F. Joel, F.T. Ademiluyi, and K. Oduola, "Performance Evaluation of Local Cassava Starches with Imported Starch for Drilling Fluid," *American J.Eng. Research (AJER),* vol. 5 no 10, pp. 111-120, 2016.

[14] E.N. Wami, C.G. Nmegbu, and J. Pius, "Drilling mud formulation using potato starch (Ipomoea Batatas),"*International J. Eng. Research and Applications*, vol. 5no 9, pp. 48-54, 2015.

[15] A. Mohammed, "Environmentally friendly mud additives for superior drilling fluid design to meet the current and future technical and environmental challenges," *The Oil and Gas Review,* Issue 11, 2007.

[16] C. Rabson, R. Chikoti, M. Mpundu, T. Mathias, and S. Peter," Cassava mosaic disease: a review of a threat to cassava production in Zambia," *J. Plant Pathology*, vol. 101, pp. 280-288, 2019.

[17] T. Ademiluyi,J.F. Ogbonna, and A.K. Amuda, "Investigation of local polymer (cassava starches) as a substitute for imported sample in viscosity and fluid loss control of water-based drilling mud," *ARPN J.Eng. and Applied Sciences*, vol. 6 no 12, pp. 43-48, 2011.

[18] NOGIC ACT, "Nigerian Oil and Gas Industry Consultative Act," Federal Republic of Nigeria, Abuja, Nigeria, 2010.

[19] U. Ibekwe, C. Eze, P. Onoh, and C. Nwajiuba, "Determinants of adoption of improved cassava production technologies among farmers in Enugu State of Nigeria: Global Approaches to Extension Practice," *A J. Agric Extension*, vol. 2 no 1, pp. 37-44, 2006.

[20] American Petroleum Institute 13A,"Specification for drilling fluid materials."American Petroleum Institute, Production Department, Bellevue, USA, 2010.

[21] FDA,"US Food and Drugs Administration Guidance on the Preparation of Investigational New Drug Products (Human and Animal).A Publication of the U.S. Department of Health and Human Services Food and Drug Administration, 1991.

[22] R. Caenn, H.C. Darley, and G.R. Gray, "Composition and Properties of Drilling and Completion fluids," 7th ed. Houston, Texas, USA, Gulf Professional Publishing, 2016.

[23] American Petroleum Institute RP 13B1, "Recommended Practice for field testing water-based drilling fluids," American Petroleum Institute, Bellevue, USA, 3rd edition, 2004.

[24] O.K. Dankwa, P. Opoku Appau, and E. Broni Bediako, "Evaluating the effects of monovalent and divalent salts on the rheological properties of water-based mud," *The Open Petroleum Eng. J.l*, vol.11 no1, pp.98-106 2018.

[25] A.A. Sarah, and S.O.Isehunwa, "Temperature and Time-Dependent Behaviour of a Water Base Mud Treated with Maize (Zea mays) and Cassava (Manihotesculanta) Starches," *J. Scientific Research & Reports*, vol. 5 no 7, pp. 591-597, 2015.

[26] E. Forssberg, M. He, and Y. Wang, "Slurry rheology in wet ultra-fine grinding of industrial minerals: a review," *Powder Technology*, vol. 147, pp.267-304, 2004.

[27] Hilfiger, M.G., Thaemlitz, C.J. & Moellendick, E. (2016). Investigating the chemical nature of flat rheology. In: *SPE Deepwater Drilling and Completions Conference*. Society of Petroleum Engineers, September, Houston, Texas.

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