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Active Fractions of Methanol Crude Obtained from *Acacia Seyal Gum* and their Anti-Proliferative Effects against Human Breast Cancer Cell Lines

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Active Fractions of Methanol Crude Obtained from *Acacia Seyal Gum* and their Anti-Proliferative Effects against Human Breast Cancer Cell Lines

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Results: The most anti-proliferative effect was detected in the sample collected from Prebio-T-commercial (IC50=8.97µg/mL) as compared to *Acacia seyal* gum (IC50=9.56µg/mL). Regarding total phenolic content (TPC), the methanol crude extracts values are 694±2.58mg, GAE/100g for Prebio-T-commercial as compared to 155.78±2.58, GAE/100g for *Acacia seyal* gum. However, both acetone and methanol fractions of *Acacia seyal* gum and Prebio-T-commercial were found to be highly anti-proliferative to human breast cancer. For bioactive compounds determinations, the methanol crude extract from *Acacia seyal* gum is mainly dominated by Isovitamin C (42.37%), Crypton (5.86%), and Hydroquinone (4.86%) as major components. Conclusion: Finally, the antioxidant and anti-proliferative properties of the active fraction have shown some evidence regarding its use in traditional medicine as well as the prevention of cancer cell growth. This suggests the potential use of their bioactive compounds as natural anticancer agents.

Keywords: *cytotoxicity activity; acacia seyal gum; breast cancer(MCF-7); methanol extract/fraction, and GC-MS/MS.*

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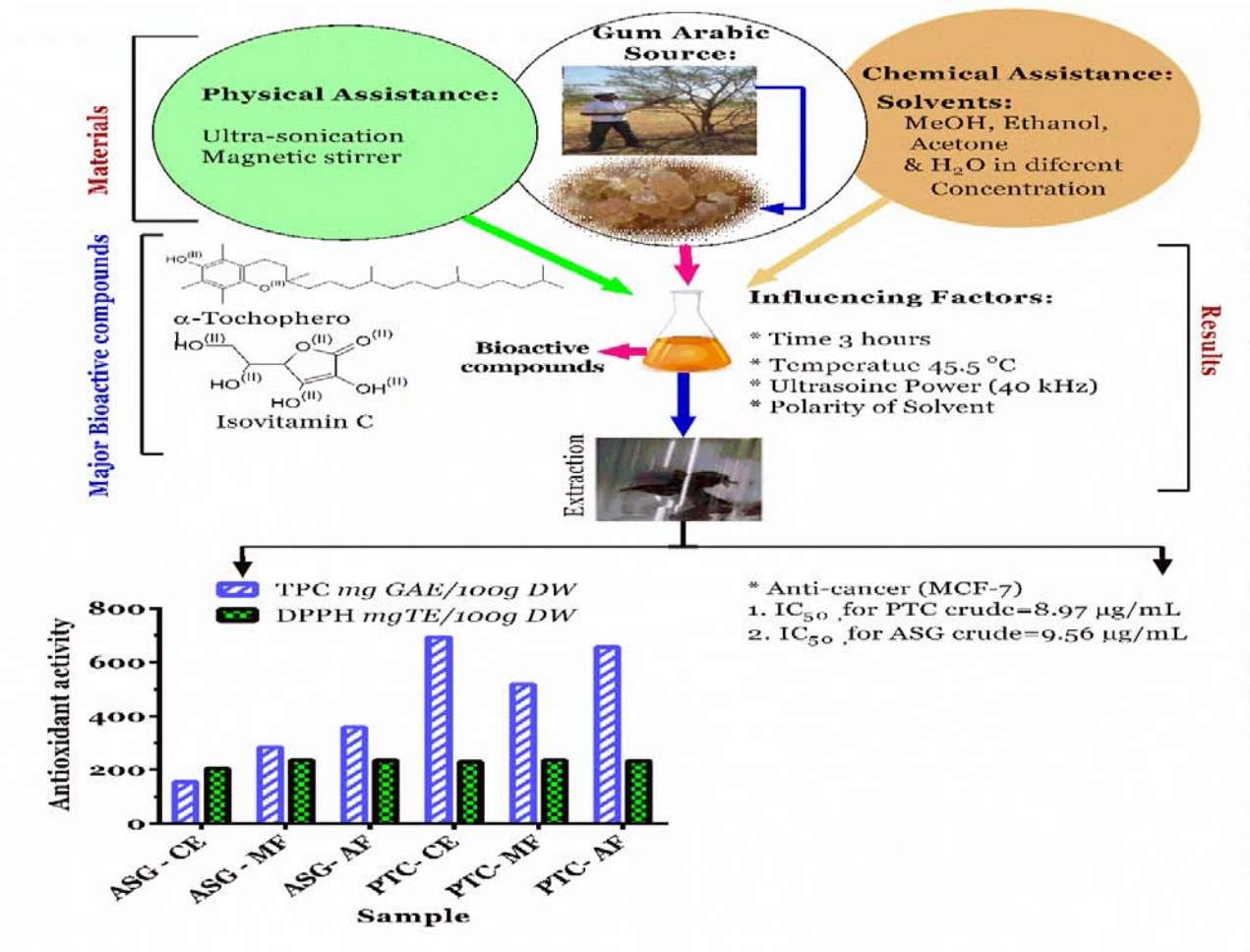
Graphical Abstract



I. Introduction

Breast adenocarcinoma (MCF-7) cell line is one of the most frequently diagnosed cancer in women globally. American Cancer Society (ACS) reported about 268,600 new cancer cases corresponding to a 30 % increase in 2019,whereas 41,670 is the estimated deaths in the USA only[1]. The World Health Organization (WHO) estimated that 84 million people would die from cancer between 2005 and 2015[2]. Thus, it constitutes a public severe health problem in both developed and developing countries.

Current protocols of treatment include radiation therapy, surgical intervention, and chemotherapy, which induce numerous side effects such as nausea, fatigue, vomiting, weak of the immune system and hair loss. Nowadays, treatment for breast cancer (BC) involveshormonal therapy, chemotherapy, surgical intervention, and radiotherapy. Exhaustive treatment with chemotherapy or radiotherapy is frequently related to few side effects ranging from the failure of bone marrow, fatigue, hair loss, vomiting, nausea, and weakness of the immune system[3]. Hence, clinical treatment remained a challenge, and new natural bioactive compounds are urgently needed. Furthermore, cancer cells are regularly not responding to chemotherapy [4]. Consequently, polyphenols from *Acacia seyal* gum (ASG) might be a potential anticancer agent in the future.

Some studies have confirmed that polyphenolics isolated from ASG components are potent biological activities with anti-inflammatory capability[5],aimed at kidney failure treatment[6], focused on a cure for cardiovascular disease[7] and also relieving gastrointestinal diseases [8] have also reported. The most abundant bioactive compounds of ASG are phenolic acids, flavonoids, terpenoids, lignans, tannins, quinones, coumarins, and alkaloids [9].Even though the anti-proliferative effect of ASG compounds was studied on several biological activities, it has not been reported in any cancer cell lines, including breast cancer (MCF-7). In this study, we focused on the cytotoxic effect of MCE and the effect of its active fraction on breast cancer cell lines. Finally, the GC-MS/MS analysis was employed for the quantification of bioactive compounds, which were thought to be responsible for the cancer treatment.

II. Materials and Methods

a) Chemicals and reagents

Folin-Ciocalteu (FC) phenol reagent and Sodium carbonate were obtained from Merck (Darmstadt, Germany) and RDH (Germany), respectively. Moreover, Trolox, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid, were from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, n-Hexane, Acetone, and Methanol were obtained through the fractionation process. All chemicals and reagents used in the study were of analytical grade.

b) Extract preparation and solvent-guided of methanol crude extract

The primary raw material was *Acacia seyal* gum (ASG), it was obtained from Blue Nile state (Sudan), in the year 2015whereas Prebio-T commercial (PTC) was obtained from Perfect Life Food company locatedin Dubai-U.A.E. Spectrophotometer (Spectro-Star Nano) was used for recording the samples'absorbance readings. 500grams of the mechanical ASG powdered was extracted with methanol by using optimized ultrasonic parameters for 3 hrs at a power of 40 kHz, and 42.5^{0}C. Chloroform, n-Hexane, Acetone, and Methanol were used in the fractionation process according to the method reported by Elnour *et al.*[5], as presented in Figure 1. The extract was concentrated and dried under nitrogen gas supply at room temperature. Finally, the extract and its active fractions were stored at 4^{0}C.

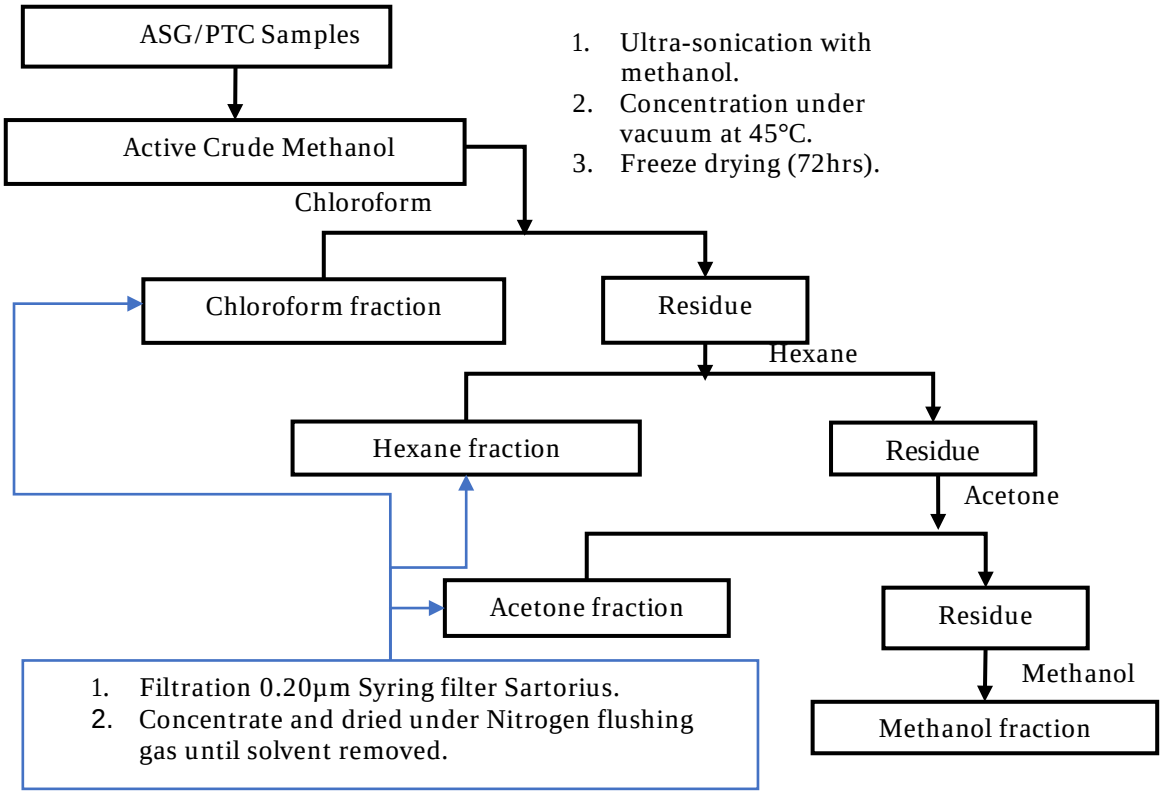


Figure 1: Schematic flow representation of the Kupchan solvent-solvent partitioning of a methanolic crude extract of *Acacia seyal* gum (ASG) and PTC crude methanol and its fractions

c) Cell lines and Culture Conditions

In this research, breast cancer MCF-7 cell lines were used as *in-vitro* experimental cancer cells (ATCC N: HTB-22™). These cells were purchased from American type Collection Culture (ATCC). Frozen MCF-7 cells were thawed and inoculated into 5 mL of RPMI 1640 medium, enhanced with 10% fetal bovine serum (FBS) and supplemented with 100 µg/ml streptomycin and 100 U/ml penicillin. The MCF-7 cells were cultured in T-25 flasks and incubated at 37°C in 95% humidified incubator with 5% CO_2. The cells were used for further experiments after reaching 70 % confluency.

i. *Anti-proliferative effect of methanol extract of Acacia seyal gum*

Were determined in the *in-vitro* cytotoxic activity of the methanol crude extract (MCE) and its active fractions against MCF-7 cell lines were determined using sulphorhodamine assay (SRB assay) as previously described by Samarakoon et al[10];however, a slight modification was done in the procedure. Briefly, cells were trypsinised and inoculated (5x10^3 cells/well) into 96-multiwell plates then incubated for 24 hours. After that, different concentrations of the compound under test (0, 1, 2.5, 5, and 10 µg/mL) were

administered to the cell wells and incubated for 48 hours. Moreover, Taxol was used as a positive control as well as DMSO at (01% v/v) served as a negative control. The cells were placed on the ice-cube with 50% trichloroacetic acid solution and incubated for 60min at 4°C, then washed five times with tap water before marking them with sulforhodamine-B stain. After that, cells were stained with 100 µL of 0.4% SRB solution/well for 15 min at room temperature. The acetic acid solution (1%) was used to remove the unbound dye, and then unbuffered. On the other hand, a Tris-based solution was utilized to solubilise the bound SRB dye. In the end, the plates were shaken for 1 hour at room temperature then the readings were recorded by a microplate reader at a wavelength of 540 nm. The obtained results were used to calculate the percentage of cell viability, and fifty percent of inhibitory concentration (IC_{50}) of each extract was determined, using equation 1[11] as follows:

$$\%\, cellviability = \frac{mean\ OD\ of\ extract\ wells}{mean\ OD\ of\ control\ wells} \times 100$$

d) Antioxidant activities of methanol extract of Acacia seyal gum

i. *Total phenolic content (TPC)*

The procedure adopted follows the method described by Musa et al. [12], whereby approximately 0.5 mL diluted Folin-Ciocalteu (FC) reagent was added to 100 µL of ASG sample. The extraction procedure was conducted using 1.0 g sample of ASG, and 10 mL of solvent for 5 min beforeadding1 mL (7.5%) of sodium carbonate (w/v). The absorption spectrophotometer of wavelength 765 nm was used after 2 hours for analysis. Gallic acid was presumed as the standard, and the results were reported as mg gallic acid equivalents to mg GAE/100g of sample dry weight (DW).

ii. *DPPH free radical scavenging assay*

Following Musa et al., [12] discussions, DPPH was freshly prepared by dissolving 40 mg DPPH in 1000 mL methanol to obtain the absorbance of 1.00±0.01 at 517 nm wavelength using a spectrophotometer, however, with slight modification. Also, 100 µL of the sample was mix with 1 mL DPPH solution and kept closed in the dark for 2 hours. On the other hand, Trolox was taken as the baseline, and the results were reported as mg Trolox equivalent (TE)per 100 g of dry sample (mgTE/100g of DW).

e) Phytochemical analysis using gas chromatography-mass spectrometry (GC-MS/MS)

Methanol crude extract (MCE), methanol fraction (MF), acetone fraction (AF), and active fractions were analysed using GC-MS/MS technique, according to Stankov et al.[13]. The GC-MS/MS was Agilent 7890A, GC/7000 MSD-Triple Quad (Agilent Technologies, Palo Alto, CA, USA), electron impact (EI) ionisationmode (70 eV, acquisition mass range of 50-600) and HP-5MS (integrated with cross-link 5%-phenyl methyl polysiloxane, 30mm×0.25mm, coating thickness 0.25µm) capillary column. Injector and detector temperature were set at 200^{0}C. The temperature of the oven was held at 50^{0}C for 30 min, then speed up to 250^{0}C at therate of 3^{0}C. Helium (99.99%) was used as the carrier gas witha flow rate of 1 ml/min. Diluted sample (1/100 in hexane, w/v) of 1.0 µl were injected. The identification of bioactive compounds depended on varies comparison. Their mass spectra (MS) is compared with those of Wiley 7N (392,086 bioactive compounds spectra), NIST 2011(contains 300,234 compounds spectra), EPA/NIH mass spectral libraries and retention times (RT).

f) Statistical analysis

The cell viability calculation was performed in triplicates. Moreover, each resulting point indicates the overall average of at least three independent trials. The results were examined and expressed in terms of the mean of the samples as well as the standard deviation. Graph Pad Prism Version 7.00 (Inc., La Jolla, CA, USA) and Minitab Software version 17® were used to calculate the statistical parameters. Finally, one-way ANOVA and Dunnett's t-test were used to identify any significant differences between the means of several independent samples.

III. Results and Discussion

a) Anti-proliferative effect of methanol extract of Acacia seyal gum

Table 1 and Figure 2-3 present the results of the anti-proliferative effect (APE) for the methanol crude extract (MCE) *Acacia seyal* gum (ASG), and PTC samples and their active fractions against the human breast tumor MCF-7 cell lines. To the best of the Author's knowledge, this is the first time for the APE of *Acacia seyal* gum investigated using MCE on *in vitro* cell lines based. In this experimental study, the human breast adenocarcinoma (MCF-7) cell lines were studied comprehensively, and therefore, the results in Figure 2 shows active crudes of methanol and acetone extraction, as well as their active fractions from both ASG and PTC.

Also, to the American National Cancer Institute (NCI) guidelines, which have also been mentioned by Fouché et al. [14], SRB assay was also used in this research. NCIC technique defines the mean value of the logarithm growth inhibition at 50% cell lines (GI_{50}) for MCF-7 tumor cell lines. Based on the NCI procedure, the methanol crude extract (MCE) obtained from ASG, and PTC showed an average means of log GI_{50}=0.980 and 0.944, respectively, for the MCF-7 cell lines. The NCI criteria show individual growth inhibition (GI_{50}) values indicate potent activity when log GI_{50}<0, similar to ' 'Taxol's values as elaborated by Table 1. Therefore,

Taxol is one of the most effective drugs used to inhibit cancer cell lines, thus termed as a positive control.

Despite excellent growth inhibition (GI) of cancer cells, Taxol also affects the growth of tumor cells, as illustrated by Table 1 and Figures 2. Unfortunately, non-tumor cells, for instance, the ones from the VERO cell line, were not included in this study for better comparison. In this regard, all assayed of methanolic extracts seem to be like Taxol, since toxicity to tumor cells had reached high concentrations only. For example, the methanolic crude extract (MCE) of both samples (PTC and ASG) was the most active reagent against MCF-7 cell lines, reaching IC_{50} value of as low as8.79±0.046 µg/mL and9.56±0.047 respectively. On the other hand, the mean value of IC_{10} was 1.51±0.02 and µg/mL1.81±0.012, whereas an average value of $IC_{90}$was recorded (51.08±9.02 µg/mLand50.39±6.01) for the MCF-7cell line respectively. On the other hand, gum arabic is more than emulsifier of food additive (E414), as many people thought.

However, methanol fractions (MF) of both ASG and PTC, had the lowest inhibition potential of log GI_{50}=1.315 for ASG and log IG_{50}=1.391 for PTC regarding MCF-7. In contrast, methanol crude extract (MCE) of both samples(ASG and PTC) presented moderate activity with mean of log GI_{50} = 0.980 and 0.944 respectively. The above trend is well elaborated in Table 1 and Figure 2. Therefore, the highest growth inhibition of MCF-7 cell lines was illustrated by MeOH crude rather than the active fractions.

Among the fractions, ASG methanol fraction displayed various means; IC_{50}=20.66±0.01, IC_{10}=10.87±0.13 and IC_{90}=39.27±4.13µg/mL, thus showing a slight selectivity with MCF-7 cell line. On the other hand, the PTC acetone fraction (AF) manifested moderate selectivity with MCF-7 cell lines as shown by the means; IC_{50}=18.58±0.03, IC_{10}=6.62±0.11, and IC_{90}=52.13±4.23µg/mL. Finally, by looking for all fractions, the acetone fraction (AF) for both ASG and PTC was found to be more selective with MCF-7 cell lines, indicating a mean of IC_{50}=12.17±0.08 and 18.58±0.03 µg/mL, respectively. Regrettably, no previous study shaded lights on similar results regarding gum arabic.

However, Manthey et al[15] reported that the activity of extracts with IC_{50} values lower than 10µg/mL should be recommended as healthy. Considering this new perspective, only four fractions, including methanol extract of PTC and ASG, as well as the acetone fraction of PTC and ASG, have shown significant results for the cell strains analysis as elaborated by Table 1. Therefore, inhibition growth IG_{50} is a superior measurement technique recommended by the American National Institute of Cancer. Nevertheless, some researchers have used different parameters. For example, previously, Boyd [16] claim that medicinal plant extract is usually valued as significant for in vitro cytotoxic activity when the IC_{50} value is less than 100 µg/mL. Moreover, another study by Kuete *et al.*[17], optimized limit of the activity for crude plant extracts at 50% inhibition (IC_{50}) of proliferation is less than 30µg/mL after exposure for 72 hours. For this reason, a comprehensive study has to be conducted for optimising the optimum level of the IC_{50} values for plants extracts.

In this regard, the findings of the study indicated that methanol crude extracts (MCE) showed the best cytotoxic activity against the selected cell lines as shown in Table 1, compared to other six solvent extracts. Also, the anti-proliferative activity can significantly be affected by gum processing and type of solvent partitioning.

Interestingly, both samples (ASG-AF and PTC-AF) have revealed results ($p \leq 0.5$) very significantly with MCF-7 human cell lines. For instance, PTC-AF found to be nearly doubled the mean(IC_{50}=18.58±0.03 µg/mL) of MCF-7 cell lines. In contrast, the mean value (IC_{50}=12.17±0.08µg/mL) of ASG–AF for the same cell lines is elaborated well by Figure 2. Figure3 illustrated the images of the surviving MCF-7 cells after 24 hours incubation period. Finally, it was concluded that solvent partitioning might have a positive effect on a wide range of human cell lines screening as well as characterisation.

Gum arabic (GA) is a well-known biopolymer compound with antioxidant properties, nephroprotective ability, and other effects that have been highlighted in some recently conducted studies. Its function on the lipids metabolism as well as the beneficial effect in the treatment of certain degenerative illnesses such as kidney failure, gastrointestinal [8,18], and cardiovascular [7] related diseases have also been reported. Thus, GA is considered to be one of the most effective natural products for treating serval diseases, including cancer.

However, there have been no indications about the comprehensive mechanism of GA towards the anticancer activity. Therefore, in this study, the high anticancer activity of the ASG and PTC methanol, and acetone crude extract and its different fractions may have attributed to their high gum bioactive compounds. The effectiveness of different fractions of ASG and PTC has different levels of cytotoxic activity against the MCF-7 human cell lines. Overall, the data are consistent with the traditional use of GA in the treatment of some cancer types and considered as potential sources for anticancer compounds.

For detecting the bioactive compounds (BCs), the components from all methanol crude extracts (MCE) and active fractions were determined by GC-MS/MS as described under the methods section. GC-MS/MS chromatograms for MCE and active fractions extracted through solvent partitioning with major bioactive compounds are shown in table 3. In Table 3, it can be clearly seen that the major constituents in the ASG and PTC were found to be Isovitamin C (42.37%), Crypton (5.86%), Hydroquinone (4.86%), Triacetic acid lactone

(2.67%), 2,4-Di-tert-butylphenol (2.67%), Cyanidin cation (2.05%), Apigenin 7-glucoside (1.9%), Benzoic acid (1.83%), (+)-α-Tocopherol (1.58%), Methyl catechol (1.42%), and 2,6-dimethylol-p-cresol (2.16%). However, these same components were almost doubled in PTC compared to ASG, as presented in Table 3.

Nine compounds namely; Crypton (7.83%), Chromone, 5-Hydroxy-6,7,8 l,-trimethoxy-2,3-dimethyl (7.01%), Phe-1,4-diol, 3,6-dimethyl (6.65%), Hydroquinone (5.31%), Ferulic acid(5.84%), Isopinocampheol (3.06%), Benzoic acid (2.02%), Isovitamin C (1.34%), and β-carotene (1.21%), were found to be significantly high and present in both ASG-AF and PTC-MF respectively. However, Vanylglycol, Quercetin 3-D-galactoside, Vitexin, Gengkwanin, Gallic acid, Retinoic acid, Zearalenone, '4',7-Dimethoxyisoflavone, and flavone, '4'-methoxy-6-acetyloxy, were calculated in methanol fraction (MF) only.

One of the most significant compounds detected by GC-MS/MS was flavonoids; this helps in understanding the most fundamental mechanism of action. For example, Isovitamin C was detected in methanol crude extract of ASG, and PTC (benzoic acid, Crypton, Hydroquinone, Patchoulol, Fisetin, α-Bisabolol, and resveratrol) was ubiquitous. These results suggest that flavonoids are not the only compounds affecting the anti-proliferative effect of Acacia gum extract, since they are present also in the extract with weak inhibition activity, sometimes in higher contents. Also, flavonoids do not indicate the leading role of inhibition of the MCF-7 cell line alone. Following Table 1 and Figure 2, the acetone fraction of both ASG and PTC shows no significant influence on the anti-proliferative effect. Therefore, further investigation is needed in order to understand the mechanism of action with regards to methanol extraction of gum Arabic. This will enhance the potential application of ASG in suppressing MCF-7 cell lines.

In an earlier study, the most abundant constituents present in the volatile fractions of gum arabic were not reported[19]. However, in this study, most of the identified bioactive compounds (BCs) were reported as polyphenols, hydrocarbons, phenolic acids, fatty acids, and several different constituents as clearly shown in Table 3. Various identified compounds have already been reported as pharmacologically active. For instance, iso-vitamin C, to copherol, and Resveratrol have shown antitumor activity in Hep3B hepatocellular carcinoma cells, as reported by Yiang et al[20], and anti-inflammation [21]. Thus, iso-vitamin C and Tocopherol may be considered as the main bioactive compound in ASG.

Furthermore, there are numerous reports about the effects of resveratrol on tumor suppressor gene and transcription factor (p53). For instance, it was proclaimed that resveratrol-induced apoptosis occurred only in cells expressing wild-type p53, not in p53 deficient cells [22]. These results demonstrated for the first time that resveratrol induces apoptosis through the activation of p53 activity. In another study conducted by Aggarwal et al., in 2006, showed that resveratrol inhibited proliferation of pulmonary artery endothelial cells, which correlated with suppression of cell progression through Sand G2-phases of the cell cycle and was accompanied by increased expression of p53 and elevation of the level of the Cdk inhibitor p21Cip 1/WAF1[23]. Thus, ASG extract demonstrated roughly 1% with resveratrol in some fractions alongside with flavonoids.

Several mechanisms have been proposed regarding the effectiveness of flavonoids, including the initiation of process of carcinogenicity promotion and influences on development and hormonal activities[24]. Flavonoids have a molecular mechanism of action namely; down regulation of mutant p53 protein, inhibition of heat shock proteins, tyrosine kinase inhibition, cell cycle arrest, inhibition of expression of R as proteins, and estrogen receptor binding capacity.

The p53 mutation is among the most common genetic abnormalities in human cancers. The inhibition of the expression of p53 may lead to the arrest of the cancer cells in the G2-M phase of the cell cycle. For this reason, flavonoids are found to down regulate the expression of a mutant p53 protein to nearly undetectable levels in human breast cancer (MCF-7) cell lines[25]. Tyrosine kinases (TK) are a family of proteins located in or near the cell membrane (CM). They allow transduction of growth factor (GF)and signals to the nucleus. Their expression is thought to be involved in on cogenesis via an ability to override standard regulatory growth control (RGC). Drugs inhibiting tyrosine kinase (TK) activity are thought to be possible antitumor agents without the cytotoxic side effects that are seen in conventional chemotherapy. Quercetin (detected in methanol fraction only) for example, was the first tyrosine kinase inhibiting compound tested in a human phase II trial [26].Thus, Quercetin can possess a cure for cancer cells.

Flavonoids are known to inhibit the production of heat shock proteins in several malignant cell lines, including breast cancer (MCF-7), leukemia, and colon cancer [25]. Interestingly, in this study, the authors believe that the flavonoids in ASG extract are not only responsible for inhibiting MCF-7 cells lines but also suppressing other cells, and therefore, further investigation will be needed.

Previously, it has been reported that flavanol epigallocatechin-3-gallate can inhibit fatty acid synthase (FAS) activity, and lipogenes is in prostate cancer cells, which is strongly associated with growth arrest and cell death[27]. Up regulation of FAS occurs early in tumor development and is further enhanced in more advanced

tumors[28].Thus, the role of polyphenolic compounds in curing tumors is necessary.

In the present study, the Quercetin and other phenolic acid have revealed the same values in almost all crude/fractions, as shown in Table 3. Moreover, Quercetin is well-known to produce modulators of cell cycle arrest (MCCA) in proliferating lymphoid cells (PLC). Also to its antineoplastic activity, Quercetin exerted growth-inhibitory effects on several malignant tumor cell lines in vitro. These included P388 leukemia cells, gastric cancer cells (NKN-7, HGC-27, NUGC-2, and MKN 28), colon cancer cells (320 DM), human breast cancer cells, human squamous, gliosarcoma cells, and ovarian cancer cells[25]. Markaverich et al[29]suggested that tumor cell growth inhibition (TCGI) using Quercetin may have integration with nuclear type II estrogen binding sites (EBS). This has been experimentally proved, increased signal transduction in human breast cancer (MCF-7) cell line is dramatically decreased by Quercetin when acting as an anti-proliferative agent[30].

Moreover, hydroquinone exhibit a superior ability to inhibit MCF-7 and MDA-MB-231 breast cell growth compared to the standard cisplatin [31]. The maximum consumption of phytoestrogens, involving flavonoids and other is of lavones groups, has shown important protection against prostate cancer risk[32]. It was confirmed during the oxidative stress period, cancer initiation may take place, and thus potent antioxidants show potential to combat the progression of carcinogenesis. The positive impact of antioxidant as an anticancer agent depends on its competence as an oxygen radical in activator and inhibitor[33].Therefore, diets rich in radical scavengers would diminish the cancer-promoting action of some radicals[34]. Thus, gum extracts have a promising natural inhibitor for breast cancer.

Also, Crypton and Hydroquinone are best known to have potential antifungal and antibacterial activities[35, 36]. Furthermore, long-chain unsaturated fatty acids (LCUFAs), such as triacetic acid lactone, also show higher antibacterial activity and are considered to be the essential ingredients of antimicrobial, food additives and some antibacterial activities[37]. Moreover, Calder [38] has reported a similar investigation as an anti-inflammatory agent for these compounds. Furthermore, benzoic acid, ferulic acid, and β- carotene also show anticancer and antioxidant activity [39-41]. Thus, the presence of such bioactive compounds in the gum arabic solvents is considered to play an extremely crucial role in the everyday pharmacological activities as shown by methanol, acetone crude, and its fractions. This finding turns a strong candidate for further in-depth studies about the anti-proliferative activity. Thus, the revaluation of Acacia gum (E414), as a food additive as well as an emulsifier, is exceptionally crucial.

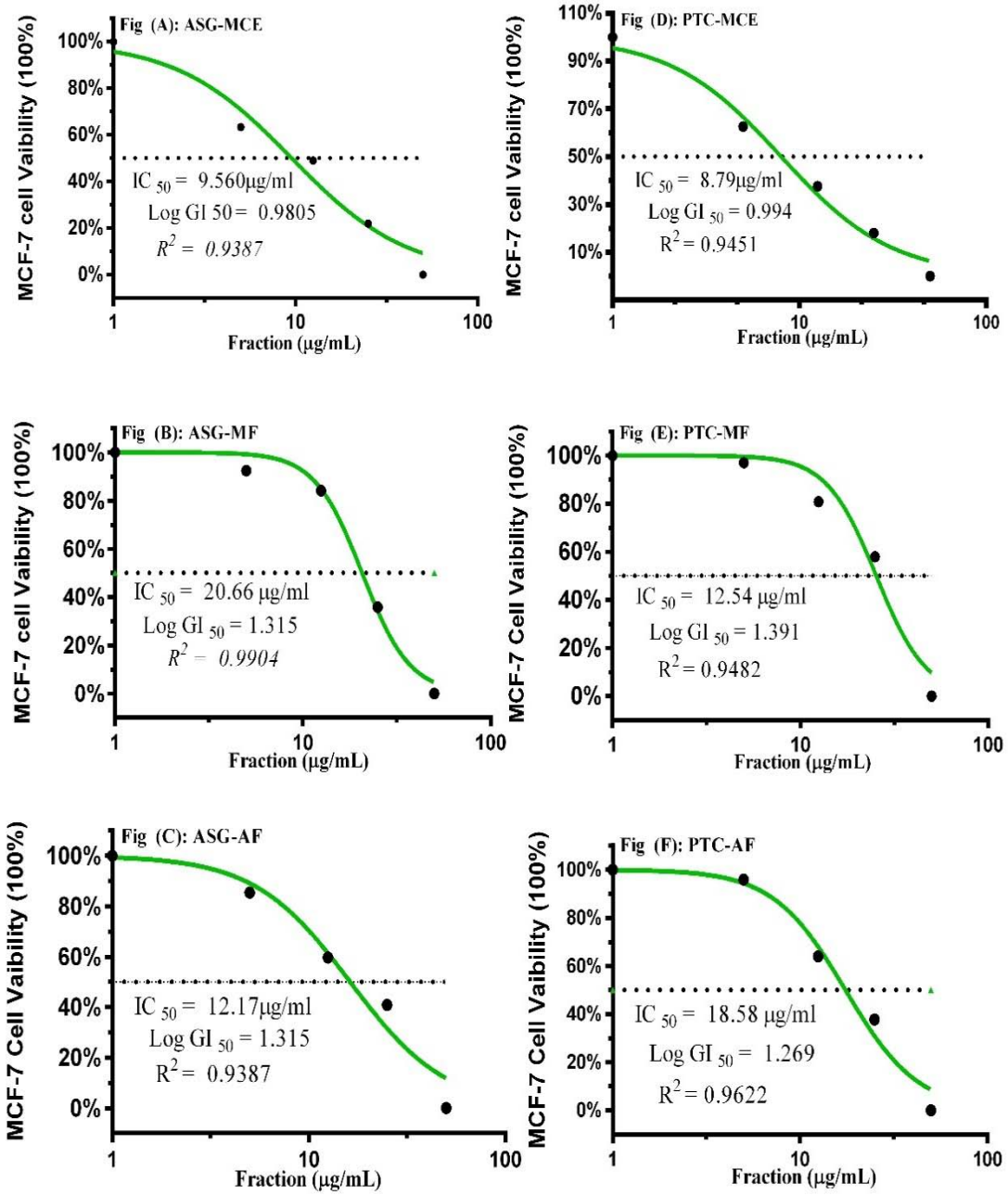


Figure 2: The percentage of MCF-7 cell viability vs. concentrations of *Acacia seyal* gum and Prebio-T commercial (PTC) methanolic crude extracts and their active fractions showed 50% cell kill against cell lines of Human Tumor Carcinoma at the concentration of µg/ml respectively. MF: Methanol fraction and AF: Acetone fraction. Data is based on triplicate experimental sets (N=3±S.D).

Table 1: Extract concentration (μg/mL) needed to 10%, 50% and 90% of growth inhibition of breast carcinoma (MCF-7) cell lines

Plant source	Active Fraction	IC10, IC50, and IC90 inhibitors of MCF-7 Cell lines a				Mean Log GI 50b
		IC50	IC10	IC90	R-square	
Taxol (+ve)[c]		0.13	0.13	0.13		-1.26 P
DMSO(-ve)[d]		0.045	0.045	0.045		
A. seyal gum raw	ASG-MCE	9.56±0.047	1.81±0.012	50.39±6.01	0.9387	0.980 M
	ASG-MF	20.66±0.01	10.87±0.13	39.27±4.13	0.9904	1.315 W
	ASG-AF	12.17±0.08	1.56±0.016	95.24±7.45	0.8343	1.085 W
Prebio-T *A.seyal* gum Commercial	PTC-MCE	8.79±0.046	1.51±0.02	51.08±9.02	0.9451	0.944 M
	PTC-MF	24.54±0.03	11.35±0.13	53.05±5.19	0.9482	1.391 W
	PTC-AF	18.58±0.03	6.62±0.11	52.13±4.23	0.9622	1.269 W

Abbr: [a]Cell lines: MCF-7: mammary.[b]National Cancer Institute criteria [14], 1: inactive, mean log GI 50>1.5;W: week activity, mean log GI 50=1.10-1.5; M: moderate activity, mean log GI 50= 0-1.10; P: potent activity, mean log GI 50=0. 'positive control'; and DMSO is 'negative control'.

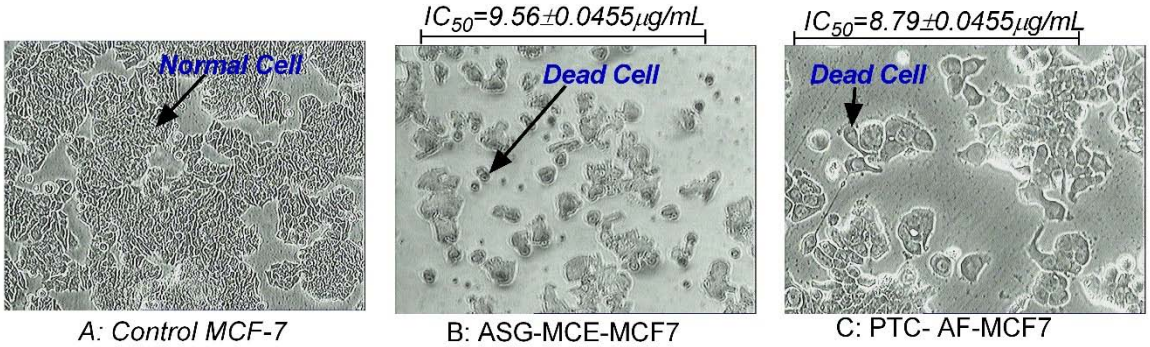


Figure 3: Demonstrative images show the potent surviving MCF-7 cells at 24 hours incubation following the treatment of *A. seyal* gum (ASG) and Prebio-T (PTC) crude extract from methanol, and their active fraction/s respectively.

b) *Antioxidant activities of methanol extract of Acacia seyal gum*

i. *Total phenolic content (TPC)*

The methanolic crude extract (MCE) of ASG and PTC samples have shown higher antioxidant activity. As presented in Table 2, the yield of extraction recorded was roughly the highest with methanol fraction (MF) instead of the acetone (AF). Between the crude extract and solvent partitioned fractions, the maximum values of the total phenolic content (TPC) was seen in the PTC samples having an average value of 694.68±3.60 mg GAE/100g DW. On the other hand, the TPC value observed was 155.78±2.58 mg GAE/100g DW for ASG samples. The TPC value of MF was found to be 285.08±3.57 mg GAE/100g DW for ASG, whereas TPC value for PTC was significantly higher ($p \leq 0.05$) at 519.93±1.64 mg GAE/100g DW. Furthermore, the TPC value of AcOH fraction (AF) was 358.57±1.58 mg GAE/100g DW for *Acacia seyal* gum (ASG) compare to the TPC value of 657.81±2.58 mg GAE/100g DW for PTC acetone fraction; this is approximately twice. The results indicated that the crude extract and solvent partitioned fractions values have a descending order of MCE, AF, then MF for PTC and AF, MF, then MCE for ASG, respectively. Both samples depicted a significant difference ($p \leq 0.05$) for antioxidant activity. Present results were in good agreement for phenolic compounds; it can be defined as a secondary metabolite with the role of antioxidants, thus owing to their capability of donating hydrogen (DH), therefore, acting as metal chelators, and quenching singlet oxygen [42]. It has been mentioned that the consumption of phenolic-rich foods or beverages prevents diseases, such as cancer, heart disease, arthritis, inflammation, immune-related diseases, neurodegenerative diseases, and diabetes [43]. This study endorsed the health benefits associated with the presence of phenolic compounds in ASG.

ii. *Antioxidant activity by DPPH assay*

The anti scavenging capacity (DPPH) was investigated for the first time as an antioxidant activity test for GA fractionation, as presented in Table 2. The maximum DPPH value was seen in methanol fraction (MF) at 235.34±1.57 mg TE/100g DW for PTC, in contrast to ASG at 235.35±1.51 mg TE/100g DW, this shows no significant differences. The DDPH antioxidant

activity of both acetone fractions (AFs) was also high, with AF obtained at the mean of 233.78±2.57 mg TE/100g DW and 234.85±1.57 mgTE/100g DW for PTC and ASG, respectively. Within each DPPH method, the mean values revealed significant differences between the crude extract and its fractions, which also significantly ($p \leq 0.5$) affects the antioxidant activity.

In this paper, the determined antioxidant activity is considered to be powerful, as compared to the standard gallic acid (GA), which also exhibits a strong correlation with the total phenolic content. The finding showed the possibility of the presence of polyphenolic molecules in ASG and the higher ability of polar solvents to extract them. Thus, the bulk of the solvent polarity is increased with the ability of extraction and thus reducing DPPH radical scavenging activity, especially methanol and acetone fractions. The findings are compared to results on some rice bran protein hydrolysates as reported recently by Phongthai *et al.*[44]. Since there is no enough data related to DPPH values of crude gum extract and gum fractionations, therefore, it was proposed that gum methanol crude extract and gum fractions could have anti-radical scavenging activity[45]. Thus, more studies are urgently needed regarding antioxidant assays in ASG.

Table 2: The antioxidant properties of different active fractions of *A. seyal* gum (natural exudate) (ASG) and *A. seyal* gum Prebio -T commercial (PTC) obtained after Kupchan-partitioning of the crude methanolic extract and its fractions as presented by Elnour et al.[5].

Plants	Extraction/ Fraction	Antioxidant activity of methanol crude and it is active fractions	
		TPC mg GAE/100g	DPPH mg TE/100g
A. seyal gum (ASG) (natural)	ASG Crude Extract (CE)	$155.78^{f} \pm 2.58$	$205.10^{d} \pm 1.50$
	ASG MF	$285.08^{e} \pm 3.57$	$235.35^{a} \pm 1.51$
	ASG AF	$358.57^{d} \pm 1.58$	$234.85^{ab} \pm 1.57$
A. seyal gum; Prebio - T (commercial)	Prebio-T-Crud Extract (CE)	$694.68^{a} \pm 3.60$	$229.01^{c} \pm 3.58$
	Prebio-T-MF	$519.93^{c} \pm 1.64$	$235.34^{a} \pm 1.57$
	Prebio-T-AF	$657.81^{b} \pm 2.58$	$233.78^{b} \pm 2.57$

Abbr: ASG-MCE: Crude Methanol Extraction, ASG-MF: methanol fraction, ASG-AF: acetone fraction, ASG-HXF: hexane fraction, and ASG-CHF: chloroform fraction, respectively. Total phenolic content (TPC) expressed as milligram Gallic acid equivalent per 100 grams dry weight of crude/fraction of sample (mg GAE/100g of crude or fraction Dry weight), and DPPH as anti-scavenging capacity expressed in mg Trolox equivalent per 100 grams dry weight of crude/fraction of sample.

iii. *The chemical composition of the solvent extracts using GC-MS/MS analysis*

In an experimental study, the crude and active fractions were extracted from ASG and PTC by solvent-solvent partitions to determine their chemical composition using GC-MS/MS analysis. According to the author's knowledge, there are no reports yet on the GC-MS/MS analysis for gum arabic regarding extraction. Their chemical investigations show that the methanol crude extract (MCE), its methanol fraction (MF) and acetone fraction (AF) of the ASG and PTC are dominated by Isovitamin C amounting to 42 % of the total composition, among the presence of a total of 21 compounds as illustrated in Table 3. The main components in this group (ASG and PTC methanol crude extract) were Isovitamin C (42.37%), Benzoic acid (6.62%), Crypton (5.86%), 2,6-Dihydroxypurine (5.11%), Hydroquinone (4.86%), (+)-α-Tocopherol (3.89%), Thiazolidin (3.38%), Triacetic acid lactone(2.67%), Apigenin 7-glucoside (2.24%), 4-Mercapto-phenol(3.37%), and Resveratrol (0.89 %) respectively. However, similar bioactive compounds were almost doubled in PTC methanol crude extract MCE as illustrated in Table 3. Furthermore, the variation in the bioactive compounds in ASG extraction content and chemical composition is attributed to some well-known factors, including geographical location, solvent polarities, and methods of partitioning employed [46, 47]. Hence, more chemical properties of different fractions of ASG is extremely crucial. Notably, the comparison between the chemical composition of methanol crude extract and its active fraction of ASG is only estimated in the present study. Furthermore, nine compounds, Cyanidin cation (22.29%), Patchoulol (13.06%), Fisetin (5.81%), Crypton (5.90%), Hydroquinone (5.23 %), 2,6-Dimethylol-p-cresol (3.49%), (Dihydrocarvone 2.18%), Iso-vitamin C (2.1%), and Quercetin(0.84%), were found to be significantly higher and present in both ASG-AF and PTC-MF respectively. However, 5,7,3',4'-Tetrahydroxy flavone (8.4%),β-Resorcylaldehyde(2.39%) were estimated in methanol fraction (MF) only. Finally, the compounds in GC-MS/MS analysis were studied based on a comparison of the mass spectra (MS) and retention time (RT) with the references present in the NIST mass spectral library. Therefore, the presence of such components in the gum arabic solvents were thought to play a crucial role in the everyday pharmacological activities as shown by methanol, acetone crude, and its fractions.

Table 3: GC-MS/MS chromatogram of bioactive compounds in *Acacia seyal* gum and Prebio-T methanol crude extractions and their active fractions. ASG-MCE: Crude Methanol Extraction, ASG-MF: Methanol Fraction, ASG-AF: Acetone fraction, PTC-MCE: crude methanol extraction, PTC-MF: methanol fraction and ASG-AF: acetone fraction respectively [5].

		Percentage of the compound in fractions Area Sum (%)								
No	Compound	ASG/ MCE	ASG/ MF	ASG/ AF	PTC/ MCE	PTC/ MF	PTC/ AF	RT	MW	MOF g/mol
1	4-Methylcatechol	1.42	1.43	1.43	3.04	4.25	0.00	3.10	$C_7H_8O_2$	124
2	Thiazolidin	2.49	3.38	3.38	3.02	3.72	1.47	3.56	$C_{10}H_9NO_3S$	223
3	Crypton	5.86	5.90	5.90	4.23	1.24	7.83	5.30	$C_9H_{14}O$	138
4	4-Mercaptophenol	1.11	2.02	2.02	3.37	0.00	1.50	6.29	C_6H_6OS	126
5	Triacetic acid lactone	2.67	1.76	1.76	2.6	2.74		4.54	$C_{10}H_{10}O_3$	178
6	Hydroquinone	4.86	5.23	5.23	5.15	1.33	5.31	7.00	$C_6H_6O_2$	110
7	Isobornyl acetate	1.05	1.89	1.89	0.00	0.00	1.34	7.86	$C_{12}H_{20}O_2$	136
8	Apigenin 7-glucoside	1.90	1.96	1.96	2.24	0.00		8.33	$C_{21}H_{24}O_9$	420
9	Benzoic acid	1.83	0.00	0.00	6.62	0.00	1.49	8.68	$C_7H_6O_2$	122
10	2,6-Dihydroxypurine	1.48	2.26	2.89	5.11	0.00	2.02	9.12	$C_5H_4N_4O_2$	152
11	(+)-α-Tocopherol	1.52	2.89	1.64	3.89	2.81	0.00	9.23	$C_{29}H_{50}O_2$	430
12	β-Resorcylaldehyde	0.00	0.00	0.00	2.39	0.00	6.65	9.34	$C_7H_6O_4$	154
13	2,4-Di-tert-butylphenol	1.10	2.27	0.00	0.00	1.60	2.45	11.12	$C_{14}H_{22}O$	206
14	2,6-Dimethylol-p-cresol	2.16	2.1	3.49	2.63	0.00	0.00	13.56	$C_9H_{12}O_3$	168
15	Isovitamine C	42.37	42.45	2.1	24	1.81	1.34	14.23	$C_6H_8O_6$	176
16	Cyanidin cation	2.05	2.14	2.4	2.39	22.29	1.46	15.89	$C_3H_3N_3$	81
17	Fisetin	0.00	1.90	1.90	0.45	5.81	2.42	16.09	$C_{15}H_{10}O_6$	286
18	Ferulic acid	0.00	7.49	7.49	1.16	0.00	1.34	16.58	$C_{10}H_{10}O_4$	194
19	Resveratrol	2.89	0.64	0.64	0.47	0.71	0.54	16.91	$C_{14}H_{12}O_3$	228
20	β-Citronellol	0.00	1.01	1.4	0.00	0.00	5.84	16.97	C10H20O	156
21	Dihydrocarvone	0.00	2.18	2.18	0.00	4.29	0.075	17.13	$C_{10}H_{16}O$	152
22	Patchoulol	1.21	3.35	3.35	1.74	13.06	1.52	17.21	$C_{15}H_{26}O$	222
23	5,7,3',4'-Tetrahydroxy flavone	0.00	8.4	0.00	0.00	2.11	1.60	17.31	$C_{15}H_{10}O_6$	286
24	Quercetin	0.44	0.39	0.84	0.33	0.46	0.49	19.35	$C_{15}H_{10}O_7$	302

Abbr:Acacia seyal gum methanol (ASG); MCE:Methanol Crude Extract; MF: Methanol fraction; AF: Acetone fraction; PTC: Acacia seyal gum (commercial sample).

iv. *Correlation analysis between methanol crude extract and its active fraction and IC_{50} of MCF-7 cell lines*

Table 4 presents the correlation coefficients of the possible correlation between the methanolic crude extract (MCE), and its active fractions and the human breast carcinoma (MCF-7) cell lines as presented in Table (4). It also shows the correlation between the different antioxidant methods used. The DPPH and TPC exhibited a significant and positive linear correlation ($p \leq 0.05$) with MCF-7. The correlation was a decreasing order of DPPH > and TPC, respectively. These results suggested that the anti-proliferative activity express as (IC_{50} values) is more closely related to DPPH than TPC. A higher positive correlation between DPPH and antiperoxidative properties also proved that the anti-scavenging compounds were the major contributors to the anti-antiproliferative capacity of the MCE and their active fractions of each *A. seyal* gum and Prebio-T (PTC) commercial. Moreover, Pearson correlation analysis of the findings showed a significant and positive correlation between cell lines IC_{50} ($p \leq 0.05$). The highest correlation was found between TPC and MCF-7 ($r=0.656$). However, DPPH anti-scavenging capacity (DPPH) material of MCE and their active fractions

resulted in the highest correlation (r =0.976) towards MCF-7. It indicated that the bioactive compounds in the extracts that could inhibit MCF-7 cell lines and served as anti-proliferative inhibitors. Furthermore, the strong correlation between DPPH and TPC, and MCF-7 respectively cell lines suggests that the antioxidants in the methanolic crude extracts, and it is active fractions react similarly with these antioxidant assays.

Table 4: Person's correlation coefficient of methanol crude extract and its active fraction and IC_{50} of cell lines.

Variables	DPPH [a]	TPC [b]
TPC mg GAE/100g DW	0.773**	
MCF7-IC_{50} (μg/mL)	0.976***	0.656*

Note: Antioxidant activity measured in methanol crude extract and their fractions on [a]DPPH radical - scavenging activity, and [b]FCI assays, correlated with MCF-7 human cell lines, * significant and, *** highly significant at $p \leq 0.05$ and 0.01.

IV. Conclusions

In this study, methanol crude extract (MCE) and its active fraction of both ASG and PTC exhibit anti-proliferative effect against breast cancer adenocarcinoma (MCF-7) cell lines by inducing loss of cell viability via cell death, change of cell morphology, and cell cycle arrest at the G0/G1 phase. This inhibition was selective to the growth of MCF-7 cell, proposing that MCE of ASG possesses selective antitumor towards cancer cells when compared to Taxol as a positive control. It also revealed their potentiality as an antioxidant activity when calculated as Gallic acid and Trolox equivalent using TPC and DPPH, respectively. Furthermore, the MCE of ASG has inhibited MCF-7 cell growth by reducing the number of cell growth inhibition (GI). These results proposed that the MCE of ASG and PTC can be considered as a novel defence-based agent for the prevention and cure of breast cancer. More studies are urgently needed to explore the mechanism of action to peruse the therapeutic impact of ASG extract, in addition to the investigation of bioactive compounds that thought to be responsible for cytotoxicity towards breast cancer.

Conflict of Interest

The authors declare that there is no conflict of interest. The copyright for reusing Table 3 is under license of permission letter (DPL-4821).

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