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Determination of Chemical Composition, Total Flavonoid Content, Total Phenolic Content and Antioxidant Capacity of Various Crude Extracts of Manihot esculenta Crantz Leaves

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***Abstract*: *In the present study, the leaves of Manihot esculenta Crantz were extracted by various organic solvents viz. hexane, chloroform, ethyl acetate and subjected to GC-MS analysis to determine their chemical composition and antioxidant activity by DPPH assay. The GC-MS analysis of hexane extract displayed squalene (10.25%), 9-octadecenoic acid (Z)-, methyl ester (9.01%), phytol (8.71%) and hexadecanoic acid, methyl ester (8.33%) as major compounds. The major compounds present in the chloroform extract were β-amyrin (12.70%), phytol (11.35%), 1,4-methanoazulen-9-ol, decahydro-1,5,5,8atetramethyl-,[1R-(1α,3aβ,4α,8aβ,9S*)]- (10.98%), 6β-Bicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-, (9.40%) and humulane-1,6-dien-3-ol (8.53%). In ethyl acetate extract, squalene (16.39%), 9,11-dimethyltetracyclo [7.3.1.0(2.7).1(7.11)]tetradecane (12.01%) and humulane-1,6-dien-3-ol (9.58%) were reported as major compounds. The total phenolic content for hexane, chloroform and ethyl acetate was 5.35 ± 0.66 mg GAE/g, 7.52 ± 0.09 mg GAE/g and 13.47 ± 0.56 mg GAE/g, respectively. Whereas the total flavonoid content for hexane, chloroform and ethyl acetate was 0.89 ± 0.17 mg QE/g, 2.69 ± 0.09 mg QE/g and 6.66 ± 0.94 mg QE/g, respectively. The antioxidant activity of ethyl acetate extract (IC_{50} = 0.19 mg/mL) was higher than chloroform (IC_{50} = 1.39 mg/mL) and hexane extracts (IC_{50} = 1.74 mg/mL), with respect to the standard butylatedhydroxytoluene (BHT) (IC_{50} = 0.04 mg/mL).*

Keywords*: *Antioxidant activity, Manihot esculenta, Total flavonoid content, total phenolic content, DPPH assay, GC-MS.

I. INTRODUCTION

Humans have used plants for medicinal purposes since ancient times [1]. Most of the medicinal plants, which are currently used to cure diseases, were identified by the well known ancient civilisations which is Ayurveda. Ayurveda identified close to 8,000 plants which are being used successfully in the classical formulation of the Ayurvedic system of medicine [2]. Based on the survey done by the World Health Organization (WHO), 80% of world population depends on herbal medicines for their health care. Scientifically, medicinal plants naturally produce and accumulate some secondary metabolites, such as flavonoids, terpenes, alkaloids, sterols, tannins and saponins. The functionality of the medicinal plant is determined by the composition of chemicals present in it. According to Kiang et al., the phytochemical screening of 205 plants in Sabah was the earliest report regarding the medicinal plant research in Malaysia, which was done by Arthur in 1954 [3]. As Malaysia is located at the tropical rain forest area, it has become of great advantage and interest among the researchers, as they are able to identify various medicinal plants through the natural resources.

Manihot esculenta Crantz which is locally known as, cassava, is a tropical, perennial plant that easily grows up to 3-5 metres in height (Fig. 1). The leaves are deeply indented, palmate with three to seven lobes that are attached to a slender stem by long petioles. The small, greenish-yellow flowers form panicles, which turn into seed capsules that explode upon ripening to distribute their load. Meanwhile, the roots form large starchy tubers, a bit similar to sweet potato with a dark brown, fibrous covering and a white flesh, which ranges from bright to soft yellow [4]. Over five thousand varieties of cassava are known, each of which has its own distinctive qualities and is adapted to different environmental conditions [5].

There are no reported literature on determination of chemical constituents of leaves of *Manihot esculenta* Crantz *via* GC-MS as well as its antioxidant properties, in the past years. However, many researches have been done on the entire plant, mainly in the field of biochemistry. Popoola *et al.*, reported antimicrobial inhibitory properties of cassava seed oil in the inhibition of skin pathogenic

microorganisms such as Staphylococcus aureus, Propionibacterium acnes, Escherichia coli, Pityrosporum ovale and Candida albicans [6]. Zakaria et al., reported low toxicity in the in-vitro antibacterial activity and brine shrimp toxicity of Manihot esculenta extracts in which chloroform extract exhibited quite good antibacterial activity against L. moncytogenes, Vibrio cholerae, Shigella flexneri, Salmonella typhi while ethanol extract was effective against P. aeroginosa, C. diphtheria and V. cholera [7]. The research of analgesic activity of ethanolic extract of Manihot esculenta Crantz leaves in mice showed, that the extract has similar potency to that of paracetamol [8]. The study of phytochemicals, nutritive and anti-nutritive composition of Manihot esculenta tubers and leaves showed that aqueous and ethanolic extracts of raw tubers comprises alkaloids, flavonoids, tannins, anthraquinone, phlobatannins, saponins, reducing sugars and anthrocyanosides. Meanwhile, raw cassava leaves contains more crude fibre and protein as compared to raw or boiled tubers. Raw and boiled tubers and the raw leaves yielded vitamins A, C, E and minerals like calcium, magnesium, phosphorus, iron, sodium and chloride ions, whose levels were reported to decrease significantly on boiling [9]. Tsumbu et al., conducted research on the antioxidant properties. Effects on Lipoperoxidation and the study of inhibition of lipid hydroperoxides with DMPD showed interestingly good IC_{50} value and high total flavonoid content [10].

Fig. 1 Plant and leaves of Manihot esculenta Crantz (Cassava).

Phytochemicals have become popular and widely used in industries due to their countless medicinal uses [11-13]. In this study, the determination of phytochemicals of the secondary constituents in Manihot esculenta is one of the objectives that will be focused on in addition to the determination of its antioxidant capacity. Antioxidants protect the cells from the damaging effects of reactive oxygen species (ROS) which results in oxidative stress, eventually leading to cellular damage [14]. Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as DNA aging and damage, cerebral ischemia, cardiac and atherosclerosis [15-17]. The main characteristic of an antioxidant is its ability to trap free radicals. If these radicals are not scavenged effectively in time, they may oxidise and damage the crucial biological molecules such as lipids, DNA, proteins or nucleic acids and eventually resulting in abnormalities leading to disease conditions [15, 16].

II. MATERIALS AND METHODS

The materials and methods used in this work are described below.

A. Collection and Preparation of Samples

The leaves of Manihot esculenta Crantz were collected at the student's residency of Tun Fuad, Universiti Malaysia Sabah. The plant was washed, cleaned and separated from stems. The leaves were chopped and dried under shed until completely dry and grounded into fine powder [18]. The powdered sample was kept in polyethylene bags and stored at low temperature until required for further procedure.

B. Chemicals and Instruments

All chemicals were purchased from reputed firms; Fischer and Sigma-Aldrich® and used as such without further purification. 1,1-diphenyl-2-picrylhydrazyl (DPPH), BHT, gallic acid, quercetin, aluminium chloride, chloroform, methanol, ethyl acetate and

hexane. Apparatus and instruments that were used were, Thermo Scientific Multiskan™ GO UV-VIS Microplate Spectrophotometer, R-210, 5975C inert XL EI/CI MSD-Triple-Axis Perkin Elmer GC-MS Detector.

C. Preparation of Crude Extracts

Crude extracts were prepared according to our previously reported literature [19]. 300 g of *Manihot esculenta* Crantz powder was divided equally into three portions and soaked for 24 h with 400 mL of hexane, chloroform and ethyl acetate. The resulting mixture was then extracted on an orbital shaker for 48 h. The extracts were filtered and the obtained liquid and solid powder were stored in refrigerator overnight. The following day, the filtered hexane, chloroform and ethyl acetate extracts were evaporated under reduced pressure until dry. The percentage yields for each sample was recorded before the determination of chemical composition, total phenolic content, total flavonoid content and antioxidant activity.

D. Determination of chemical Composition by GC-MS analysis

The chemical compositions of various organic extracts of *Manihot esculenta* Crantz leaves were analysed by GC-MS analysis. A clarus 500 Perkin Elmer system comprising a AOC-20I auto sampler and gas chromatograph, interfaced to a mass spectrophotometer instrument, equipped with column Elite-1, fused silica capillary column (30 mm x 0.25 mm I.D x 1 µM df), operating in the electron impact mode at 70 eV was used. Inert gas, helium was used as carrier gas at a constant flow rate of 1.21 mL/min and injection volume of 0 µL (split ratio of 10:1). Injector temperature and ion-source temperature were set at 250˚C and 230˚C, respectively. The oven temperature was programmed from 100˚C (isothermal for 2 min), with an increase of 10˚C/min to 280˚C, ending with a 9 min isothermal at 280˚C. Mass spectra was recorded at 70 eV, a scan interval of 0.5 seconds and fragments from 50 to 300 Da. The total GC running time was reported to be 28 min. The diluted crude samples (1/100, v/v in appropriate solvent) were filtered. The relative percentage of the crude extract constituents was expressed as % by peak were normalized. The identification of chemical constituents of the crude extracts was based on gas chromatography retention time on VF–5 capillary columns, computer matching of mass spectra of NIST Library [20].

E. Determination of Total Phenolic Content (TPC)

Determination of total phenolics in the crude extracts was conducted by using the Folin-Ciocalteau reagent taking Gallic acid as internal standard [21]. The total phenolic content was expressed in mg of the total gallic acid, GAE mg/g of sample extract. The results were determined from the regression equation of calibration curve. To derive the calibration curve, 100 µL from 20, 40, 60, 80 and 100 µg/mL of gallic acid solution was mixed with 200 µL of 10% Folin-Ciocalteau reagent and 800 µL of 2% sodium carbonate was added to each of the solution mixture after 3 min. The mixture was then allowed to stand for 2 h in dark at room temperature. The absorbance of all samples was measured against a reagent blank at 765 nm using UV-Vis microplate spectrophotometer. The total phenolic content of the sample was determined from the calibration curve plotted using the gallic acid linear regression equation (R_2 = 0.9945) to calculate the TPC values of the sample. Equation 1 calculated the total phenolic content in the extracts in gallic acid equivalent (GAE).

F .Determination of total flavonoid content (TFC)

Aluminium chloride colorimetric method was adapted for the determination of total flavonoids as reported by Chang *et al.*, in 2002 [22]. Quercetin was used as the standard for the calibration curve. The result was obtained from the regression equation of the calibration curve of the quercetin. The levels of total flavonoids content were determined and expressed as milligram quercetin equivalent per gram of sample (QE mg/g of sample). In brief, 100 µL from 20, 40, 60, 80, and 100 µg/mL of quercetin was mixed with 360 µL methanol, 24 µL of 10% aluminium chloride, 24 µL of 1 M potassium acetate and 680 µL distilled water. After 30 min of incubation at room temperature, the absorbance against blank was obtained at 415 nm using UV-Vis microplate spectrophotometer. The total flavonoid content was calculated using the calibration curve for quercetin (R_2 = 0.9997). The result was expressed in quercetin equivalent per gram of dry weight of crude extract (QE mg/g of sample). Equation 1 calculated the total flavonoid content in the extract in quercetin equivalent (QE).

$$T = C.V/M \quad \text{Eq-1}$$

Where,

T = is the total content of phenolic compounds, mg/g plant extract in GAE

C = is the concentration of gallic acid established from the calibration curve

V = volume of extract

M = weight of plant extract in gram

G. DPPH radical Scavenging Assay

The evaluation of antioxidant activity was carried out spectrophotometrically as described by Khan *et al.,* 0.1 mM stock solution of DPPH was prepared by dissolving 2.0 mg of DPPH with 50 mL of methanol [19]. The standard solution of BHT was prepared by dissolving 10 mg of BHT with 10 mL of methanol to obtain a concentration of 1 mg/mL. The standard BHT solution was further diluted by methanol to obtain a series of different concentration of 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.25 mg/mL and 0.50 mg/mL. Further, 585 µL of previously prepared DPPH was added into each concentration and shaken vigorously. The resulting mixture was left in the dark for 60 min at room temperature. To prevent light degradation, each test tube was covered with aluminium foil. The absorbance of all the samples was recorded at 517 nm by UV–Vis spectrophotometer. Similar procedure was repeated with crude extracts at 0.125 mg/mL, 0.250 mg/mL, 0.500 mg/mL, 1.000 mg/mL, 2.000 mg/mL and 4.000 mg/mL of concentrations. DPPH assay is based on the ability of the plant extract to scavenge stable DPPH. This could be observed by the colour change of DPPH from purple to yellow. The assay was carried out in triplicates and data were reported as means and standard deviation values. The interpretation of the result was based on the inhibition activity (I%). The calculation of I% was done according to the Equation 2 and IC_{50} was calculated from the regression equation for the concentration of extract and inhibition activity (I%) by Equation 3 and the results were expressed statistically as means and standard deviation values. All data were analysed through linear regression using Microsoft Office Excel.

$$I\% = (A_{control} - A_{sample} / A_{control}) \times 100 \quad \text{(Eq-2)}$$

Where,

$A_{control}$ = Absorbance of the control reaction

A_{sample} = Absorbance in the presence of the plant extracts

IC_{50} : $y = mx + c$ (Eq-3)

III. RESULTS AND DISCUSSION

All the three organic extracts viz. hexane, chloroform and ethyl acetate were screened quantitatively via GC-MS analysis in order to identify their chemical constitutes. Further DPPH radical scavenging assay was performed to determine their antioxidant properties. Hexane extract displayed the highest yield (5.24%) followed by chloroform extract (1.89%) and ethyl acetate extract (1.20%) (Table 1). It is worth to mention here the research conducted by Ghazali and Yasin, which reported that a non-polar solvent is a better solvent for extraction which is also supported by the previous study conducted by Nwabueze and Okocha [23, 24]. This could be visible through our study by observing the percentage yield of hexane crude extract.

TABLE I

Percentage Yields Of Crude Extracts Obtained From Solid Residue Of Plant Material

Crude extracts	Crude extract weight (g)	Percentage yield (%)
Hexane	9.46	9.46
Chloroform	2.70	2.70
Ethyl acetate	1.74	1.74

Initial mass of leaves sample = 100 g

A. Chemical composition of hexane, chloroform and ethyl acetate extract by GC-MS analysis

The chemical composition of volatile compounds present in *M. esculenta* crude extract were identified through GC-MS analysis. The compound, displaying the highest peak area was chosen. The percentage in the chromatogram was shown as peak percentage area. The compounds with their molecular weight, molecular formula, retention time and peak area were obtained. The

chromatogram for each of the chosen extract are displayed in Fig. 5, Fig. 6 and Fig. 7, whereas, the full list of compounds in all the three samples are shown in Table II, Table III, and Table IV, respectively.

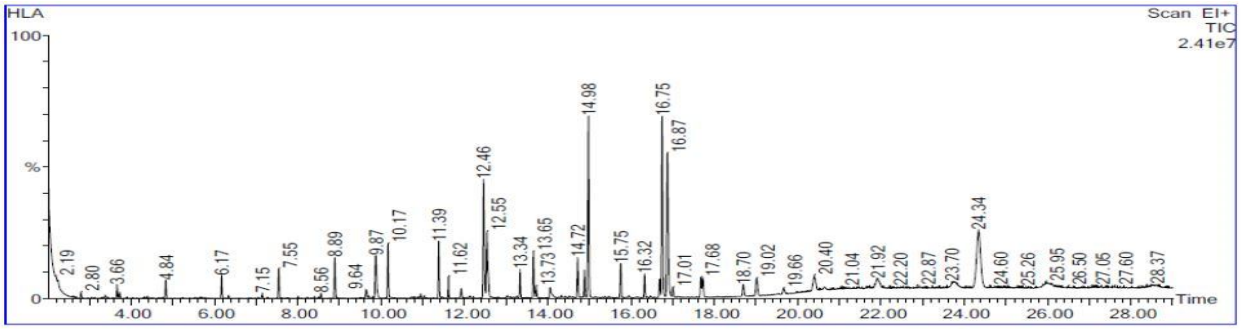

Fig. 5 Chromatogram of Hexane extract.

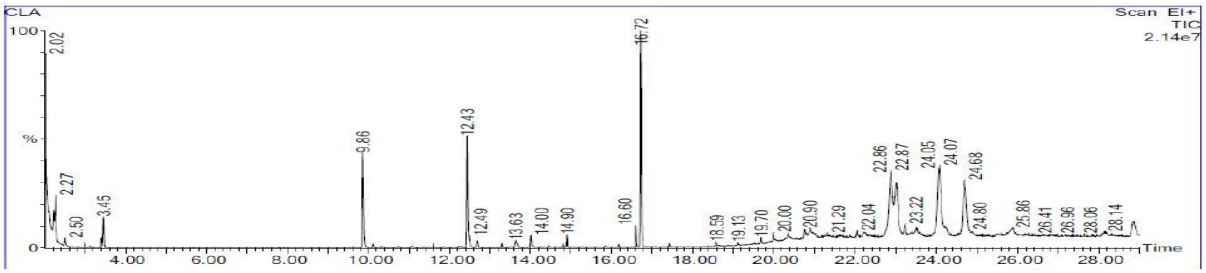

Fig. 6 Chromatogram of Chloroform extract.

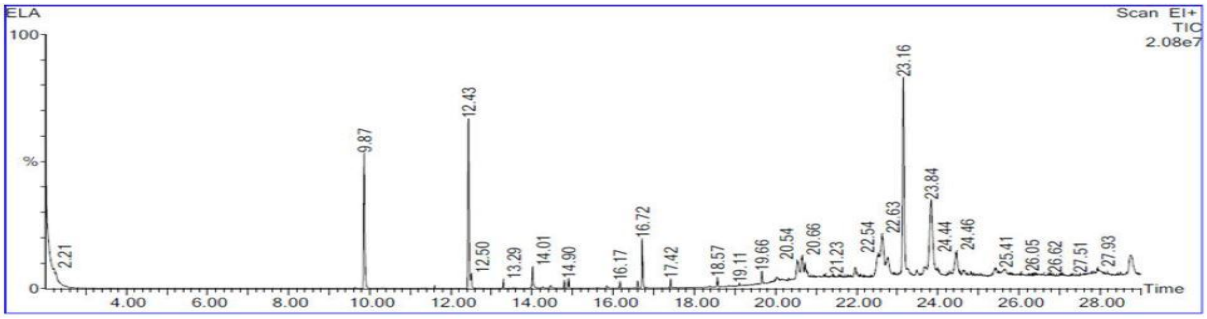

Fig. 7 Chromatogram of Ethyl acetate extract.

TABLE II

Chemical composition of hexane extract of *m. Esculenta* leaves

No.	RT (min)	Compound	MF	MW (g/mol)	Peak area (%)
1	8.89	Tetradecane	$C_{14}H_{30}$	198	1.81
2	10.167	*n*-Cetane	$C_{16}H_{34}$	226	2.22
3	11.388	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	2.28
4	12.461	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	5.49
5	12.548	Eicosane	$C_{20}H_{42}$	282	3.56
6	13.654	Sulforous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278	1.90
7	14.72	Heptacosane	$C_{27}H_{56}$	380	1.64
8	14.982	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	8.33
9	15.75	Octane, 2,7-dimethyl-	$C_{10}H_{22}$	142	2.21
10	16.752	9-Octadecenoic acid (*Z*)-, methyl ester	$C_{19}H_{36}O_2$	296	9.01
11	16.873	Phytol	$C_{20}H_{40}O$	296	8.71
12	24.343	Squalene	$C_{30}H_{50}$	410	10.25

RT=Retention time, MF=Molecular Formula, MW= Molecular weight.

TABLE III
Chemical composition of chloroform extract of m. Esculenta leaves.

No.	RT (min)	Compound	MF	MW(g/mol)	Peak Area (%)
1	2.268	3-oxetanol, 2,2,3-trimethyl-	$C_6H_{12}O_2$	116	2.216
2	3.45	Benzaldehyde	C_7H_6O	106	3.22
3	9.859	1-Dodecanol	$C_{12}H_{26}O$	186	5.338
4	12.427	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	5.669
5	16.719	Phytol	$C_{20}H_{40}O$	296	11.351
6	20.903	3,3,7,11-Tetramethyltricyclo-[5.4.0.0(4,11)]undecan-1-ol	$C_{15}H_{26}O$	222	2.224
7	22.874	1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1R-(1α,3aβ,4α,8aβ,9S*)]-	$C_{15}H_{26}O$	222	10.977
8	23.002	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	222	8.531
9	24.068	β-Amyrin	$C_{30}H_{50}O$	426	12.702
10	24.685	6β-Bicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-,	$C_{15}H_{25}I$	332	9.395
11	28.86	8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2,4-quinazolinedione	$C_{12}H_{18}N_2O_2$	222	2.56

RT=Retention time, MF=Molecular Formula, MW= Molecular weight

TABLE IV
Chemical composition of ethyl acetate extract of m. Esculenta leaves

No	RT (min)	Compound	MF	MW (g/mol)	Peak Area (%)
1	9.866	1-Dodecanol	$C_{12}H_{26}O$	186	7.75
2	12.434	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	8.71
3	16.719	Phytol	$C_{20}H_{40}O$	296	2.54
4	20.661	1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1R-(1α,3aβ,4α,8aβ,9S*)]-	$C_{15}H_{26}O$	222	2.26
5	22.626	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	222	9.58
6	22.767	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	$C_{13}H_{22}OSi_2$	250	2.75
7	23.156	Squalene	$C_{30}H_{50}$	410	16.39
8	23.840	9,11-Dimethyltetracyclo-[7.3.1.0(2.7).1(7.11)]tetradecane	$C_{16}H_{26}$	218	12.01
9	24.463	3,3,7,11- Tetramethyltricyclo-[5.4.0.0 (4,11)]undecan-1-ol	$C_{15}H_{26}O$	222	3.13
10	28.762	1-(4-Nitrophenyl)piperazine	$C_{10}H_{13}N_3O_2$	207	3.94

RT=Retention time, MF=Molecular Formula, MW= Molecular weight.

It is evident from Table II, that the major compounds present in the hexane extract, based on the peak area were squalene (10.25%), 9-octadecenoic acid (*Z*)-, methyl ester (9.01%), phytol (8.71%) and hexadecanoic acid, methyl ester (8.33%). Most of the compounds identified from hexane extract consists of hydrocarbons of monoterpenes and sesquiterpenes. Based on the report by Popa *et al.*, squalene is a triterpene, which is known to be the main component in shark liver oil, and it plays an important role as an intermediate in biosynthesis of cholesterol in organisms. Moreover, hexadecanoic acid, methyl ester ($C_{19}H_{36}O_2$) is among one of the powerful compounds possessing high antioxidant activity [25, 26]. It is worth mentioning, that these bio-active compounds are when synthetically synthesized then also they exhibit quite strong biological activities with the additional advantage of good yields [27, 28]. Hence, they could be prepared in bulk for pharmaceutical purposes.

The major compounds identified from the chloroform extract (Table III) were *β*-Amyrin with percentage peak area of 12.70%, followed by phytol (11.35%), 1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1*R*-(1*α*,3a*β*,4*α*,8a*β*,9*S**)]-(10.98%), 6*β*-Bicyclo[4.3.0]nonane, 5*β*-iodomethyl-1*β*-isopropenyl-4*α*,5*α*-dimethyl-, (9.40%) and humulane-1,6-dien-3-ol (8.53%). The application of *β*-amyrin has been identified in biological activities where it was found to exhibit the antifungal and antimicrobial

activity against certain microbes [29]. Some of the compounds do not have any reported industrial application from any journals. There is a high possibility that these compounds are from the gas chromatography column.

The major compounds found in the ethyl acetate extract as evident form Table IV, were squalene (16.39%), 9,11-dimethyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane (12.01%) and humulane-1,6-dien-3-ol with percentage peak area of 9.58%. Most of the compounds identified from this extract were monooxygenated hydrocarbons.

B. Discussion of GC-MS Data

The dynamic interaction between solute, mobile and stationary phases lead to the component zones broadening as the solute progresses along the column of the gas chromatography [30]. These kinetic processes resulted in familiar chromatographic peaks, which represent the component concentration in the mobile phase observed at the end of the column as a function of elution time [31]. It is evident from some research, that the chromatographic peaks have a complex shape [32]. In some cases, the GC-MS data might contain a range of imperfections and irregularities where this could lead to complicated interpretations. In turn, this might result in zero intensities or entire blocks of zero intensities, embedded in the data which could complicate the analysis of the noise.

There are several experimental factors that influence the characteristics of GC-MS data as what reported by Likic [31]. Firstly, the nature of sample components. Samples that are more complex produce more signals per standard chromatographic separation run and this result in increased peak crowding and overlap. Secondly, the sample matrix can profoundly influence both, the characteristics and quality of the GC-MS data. Samples of biological material can have large amounts of background chemicals, which interfere with the detection of trace compounds, both through impeding the efficacy in separation or detection and by producing noise-like effects.

Next is the condition of instrument. Less than optimal instrument condition might result in chemical noise that is difficult to model. For example, a worn out liner, a component of the GC inlet system, may deform peak shapes and affect peak resolution. Mechanical problems associated with gas chromatography, such as uneven flow of the carrier gas or column packaging might have similar effects. Other factor is the instrument tuning and experiment runtime parameters. The parameters set by the operator, if not optimal, might adversely affect the quality of GC-MS data. As an instance, faster oven ramp rates results in shorter experimental time, but also increases peak crowding and consequent peak overlap. Data acquired on different GC-MS instruments might have different characteristics such as retention time resolution, *m/z* resolution and noise characteristics.

C. TPC in the leaves of Manihot Esculenta Crantz

As evident from Fig. 8, ethyl acetate showed the highest concentration of TPC which was 13.47 ± 0.56 mg GAE/g, chloroform displayed TPC of 7.52 ± 0.09 mg GAE/g and hexane displayed TPC of 5.35 ± 0.66 mg GAE/g. The percentage difference between ethyl acetate and chloroform was calculated to be 44.17%, while between chloroform and hexane it was 28.83% and between ethyl acetate and hexane was 60.27%. Based on the percentage, it was concluded that there was significant difference in the amount of TPC between the three crude extracts. The results showed that ethyl acetate extract displayed a higher phenolic content. This was because of the fact that phenolics are well extracted in polar solvents for example ethyl acetate (Table V). The phenolic extracts of a plant is always a mixture of different classes of phenols, which are selectively soluble in different solvents. The use of an alcoholic solution and the mixture of alcohol and water provides satisfactory results for the extraction process and also has the advantage of modulating the polarity of alcoholic solvents [33, 34]. The calibration curve of Gallic acid is shown in Figure 8 and the TPC is displayed in Fig. 9.

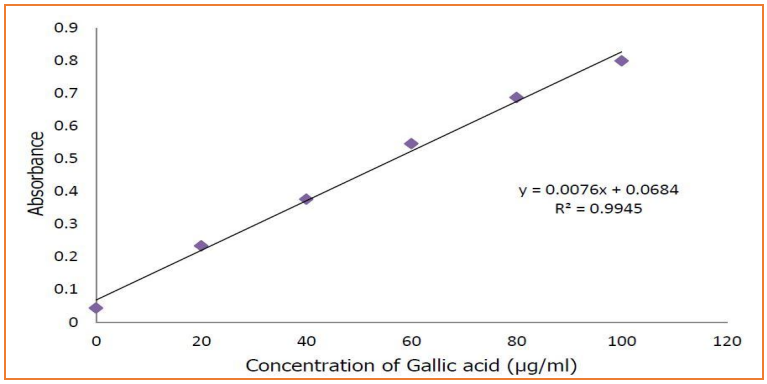

Fig. 8 Calibration curve of Gallic Acid

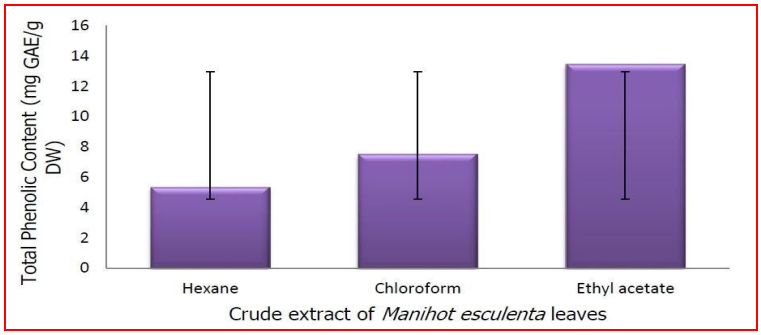

Fig. 9 Total Phenolic Content of Manihot esculents Crantz leaves

D. TFC in the leaves of Manihot esculenta Crantz

As it is evident form Fig. 12, the TFC in ethyl acetate extract is the higher (6.66 ± 0.94 mg QE/g) than chloroform extract (2.69 ± 0.09 mg QE/g) and hexane (0.89 ± 0.17 mg QE/g) (Table V). The percentage difference between ethyl acetate and chloroform was 59.55%, while between chloroform and hexane was 68.51% and between ethyl acetate and hexane was 86.65%. Fig. 10 and Fig. 11 displays the calibration curve for quercetin and the TFC of the organic extracts.

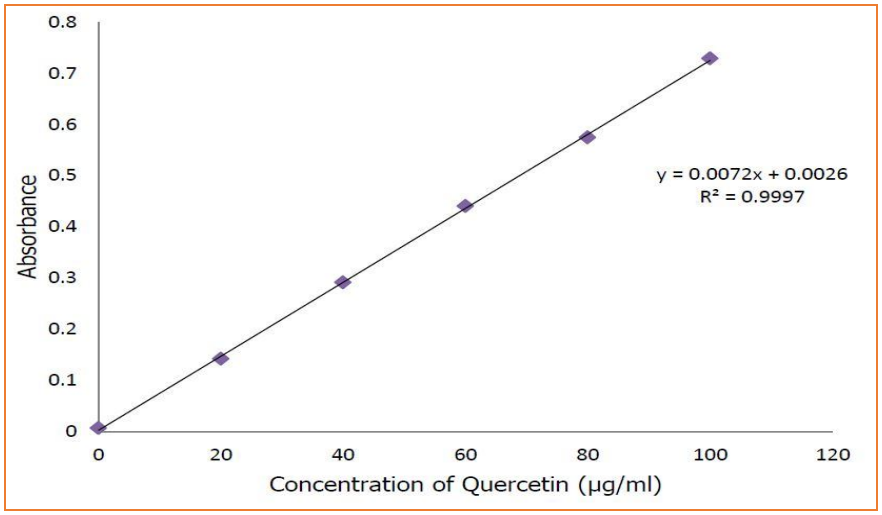

Fig. 10 Calibration curve of Quercetin.

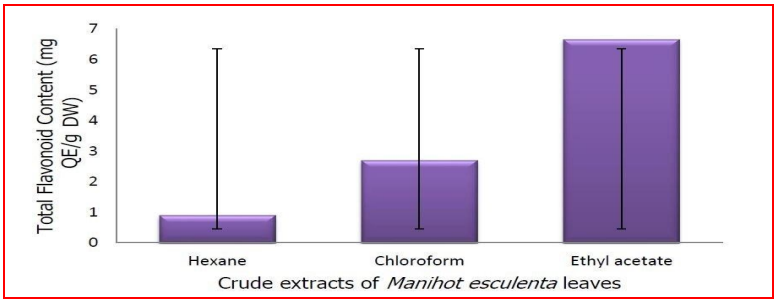

Fig. 11 Total Flavonoid Content of Manihot esculents Crantz leaves

E. Determination of antioxidant activity of various organic extracts of Manihot esculenta Crantz

The antioxidant activity of all the crude extracts was determined by DPPH radical scavenging assay using BHT as an internal standard as reported by Khan *et al.*, [19]. The reduction of DPPH radical was determined by the decrease in its absorbance at 517 nm, induced by antioxidants, due to the reaction between antioxidant molecules and radicals, which in turn results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour form purple to yellow [35]. In the present study, the interpretation of the result was based on the inhibition acitivity (I%), from which IC_{50} value was evaluated by the regression

equation for the concentration of extract and inhibition activity (I%). Table 5 shows the IC_{50} value of BHT and various *M. esculenta* extracts. Standard BHT has the lowest value of IC_{50}. This mean, BHT possesses the highest antioxidative properties with IC_{50} value of 0.04 µg/mL. Among the crude extracts, ethyl acetate displayed the lowest IC_{50} of 0.19 µg/mL, followed by chloroform and hexane with IC_{50} value of 1.39 µg/mL and 1.74 µg/mL respectively. IC_{50} is the concentration at which radical DPPH concentration were oxidized by 50%. The antioxidant properties are said to get increase with decreasing IC_{50} value. Therefore, ethyl acetate extract has the highest antioxidative properties compared to hexane and chloroform extracts.

TABLE V
Ic_{50} value, tpc and tfc of various organic extracts of *m. Esculenta* leaves

Sample	IC_{50} (mg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)
Butylated hydroxytoluene	0.04	----	---
Hexane	1.74	5.35 ± 0.66	0.89 ± 0.17
Chloroform	1.39	7.52 ± 0.09	2.69 ± 0.09
Ethyl acetate	0.19	13.47 ± 0.56	6.66 ± 0.94

Fig. 12 shows the inhibition activity of BHT as a standard, showing the inhibition activity with the maximum value of 88.84%, meanwhile Fig. 13 shows the inhibition activity of hexane, chloroform and ethyl acetate extracts. Based on the Figure 14, the antioxidant activity of each crude extract increases with concentration. Among the results, ethyl acetate extract has the highest inhibition activity where the maximum inhibition is 97.35%. Meanwhile, chloroform has the inhibition activity of 69.14%. Hexane has the lowest inhibition activity with maximum inhibition of 63.07%, which is slightly lower than chloroform. Fig. 14 shows the bar chart comparison of IC_{50} values of different samples. It is obviously clear from the bar chart that the standard BHT has the lowest value of IC_{50} whereas, hexane extract has the highest IC_{50} value.

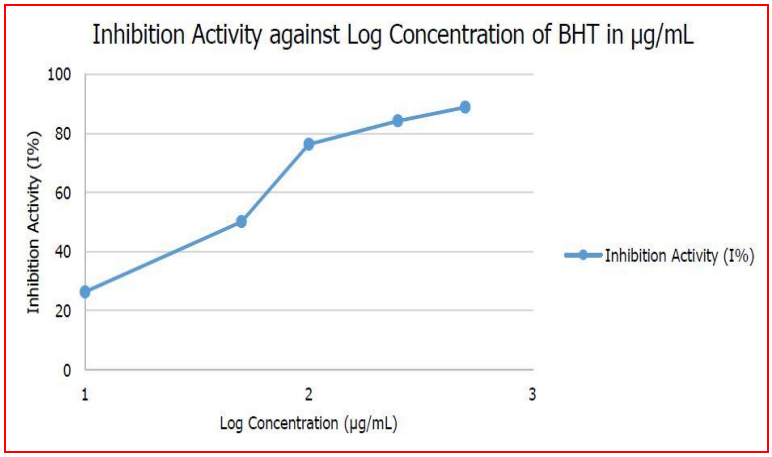

Fig. 12 Inhibition activity (I%) against log concentration (µg/mL) of BHT

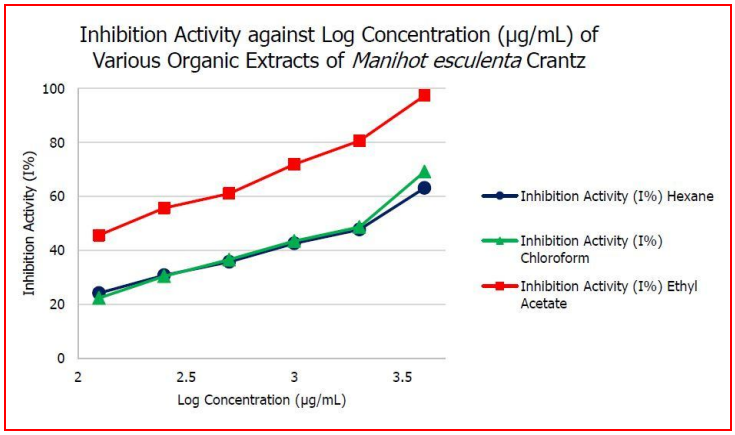

Fig. 13 Inhibition activity (I%) against log concentration (µg/mL) of various organic extracts of *Manihot esculenta* Crantz.

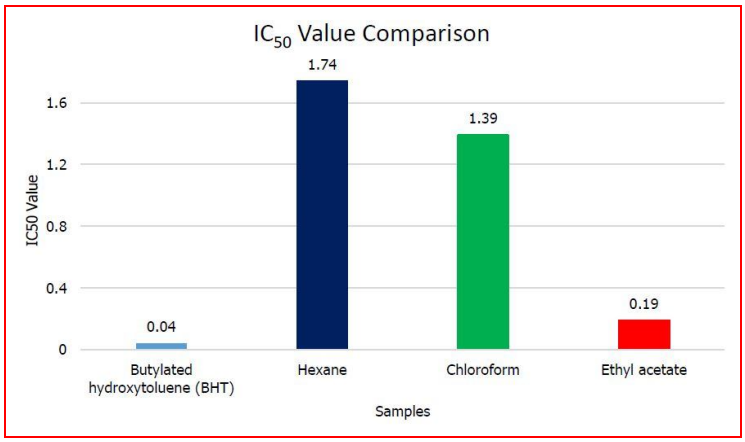

Fig. 14 The IC_{50} value for standard BHT and various organic extracts of Manihot esculenta Crantz.

IV. CONCLUSIONS

The leaves of Manihot esculenta Crantz were extracted with methanol and rinse accordingly to their polarity with hexane, chloroform and ethyl acetate. The crude extracts were analysed qualitatively using GC-MS to determine the chemical composition of each crude extract. The compounds were chosen based on the higher percentage peak area. In hexane extract, a total of 12 compounds were identified. The major compounds present in the hexane extract were squalene (10.25%), 9-octadecenoic acid (Z)-, methyl ester (9.01%), phytol (8.71%) and hexadecanoic acid, methyl ester (8.33%). 11 compounds were identified in the chloroform extract where the compounds with the highest peak area were β-amyrin (12.70%), phytol (11.35%), 1,4-methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1R-(1α,3aβ,4α,8aβ,9S*)]- (10.98%), 6β-bicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-, (9.40%) and humulane-1,6-dien-3-ol (8.53%). 10 compounds were identified from ethyl acetate extract where the major compounds were squalene (16.39%), 9,11- dimethyltetracyclo[7.3.1.0(2.7).1(7.11)]tetradecane (12.01%) and humulane-1,6-dien-3-ol with percentage peak area of 9.58%. Analysis of TPC and TFC showed that the ethyl acetate extract contained its highest amount as compared to hexane and chloroform extracts, which was 13.47 ± 0.56 mg GAE/g and 6.66 ± 0.94 mg QE/g, respectively. The antioxidative properties of each crude extract was carried out via DPPH radical scavenging assay using BHT as a positive control. The IC_{50} value of BHT was 0.037 mg/mL with maximum inhibition activity of 88.84% at the concentration of 0.50 mg/mL. Among the crude extracts, ethyl acetate extract displayed the highest percentage of inhibition activity which was 97.35% at the concentration 4.0 mg/mL and the lowest IC_{50} value which was 0.19 mg/mL. Chloroform displayed the inhibition activity of 69.14% with IC_{50} value of 1.39 mg/mL. The inhibition activity of hexane extract at 4.0 mg/mL was 63.07% which was the lowest than the others with IC_{50} value of 1.74 mg/mL. None of the crude extract could exceed the IC_{50} value of BHT. This shows that ethyl acetate extract possess the highest antioxidative properties. Therefore, this plant could be considered as a good natural source of phytochemicals, which could provide precious functional ingredients and could be used for the prevention of diseases related to oxidative stress in the human body.

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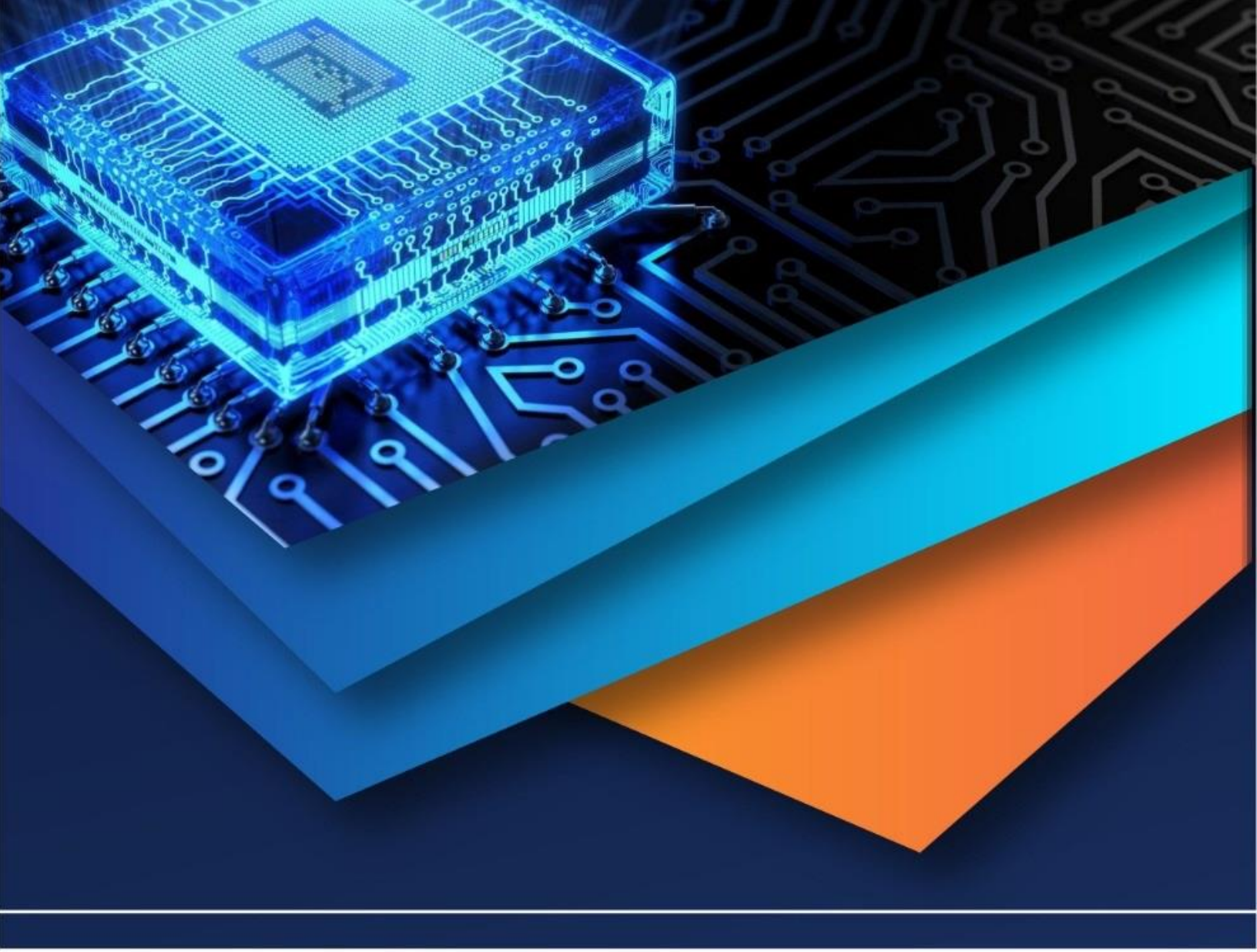
REFERENCES

[1] Petrovska, B. B., and Cekovska, S. Extracts from the history and medical properties of garlic. Pharmacognosy reviews, vol. 4, pp. 106-110, 2010.
[2] Shukla, N. Role of Ayurveda in emergency treatment. Emergency Medicine, vol. 2, pp. 1-5, 2012.
[3] Kiang, A. K., Douglas, B., and Morsingh, F. A PHYTOCHEMICAL SURVEY OF MALAYA: Part II. Alkaloids. Journal of Pharmacy and Pharmacology, vol 13, pp. 98-104, 1961.
[4] Roa, A. C., Maya, M. M., Duque, M. C., Tohme, J., Allem, A. C., and Bonierbale, M. W. AFLP analysis of relationships among cassava and other Manihot species. Theoretical and Applied Genetics, vol. 95, pp. 741-750, 1997.
[5] Clement, C. R., de Cristo-Araújo, M., Coppens D'Eeckenbrugge, G., Alves Pereira, A., and Picanço-Rodrigues, D. Origin and domestication of native Amazonian crops. Diversity, vol. 2, pp. 72-106, 2010.

[6] Popoola, T. O. S., Yangomodou, O. D., and Akintokun, A. K. Antimicrobial activity of cassava seed oil on skin pathogenic microorganisms. Res J Med Plant, vol. 1, pp. 60-64, 207.
[7] Zakaria, Z. A., Khairi, H. M., Somchit, M. N., Sulaiman, M. R., Mat Jais, A. M., Reezal, I., Mat Zaid, N. N., Abdul Wahab, S. N. Z., Fadzil, N. S., Abdullah, M. and Fatimah, C. A. The in vitro antibacterial activity and brine shrimp toxicity of Manihot esculenta var. Sri Pontian extracts. Int J Pharmacol, **vol.** 2**, pp.** 216-220, 2006.
[8] Miladiyah, I. Analgesic activity of ethanolic extract of Manihot esculenta Crantz leaves in mice. Universa medicina, vol. 30, pp. 3-10, 2016.
[9] Ebuehi, O. A. T., Babalola, O., and Ahmed, Z. Phytochemical, nutritive and anti-nutritive composition of cassava (Manihot esculenta L) tubers and leaves. Nigerian Food Journal, vol. 23, pp. 40-46, 2005.
[10] Tsumbu, C. N., Deby-Dupont, G., Tits, M., Angenot, L., Franck, T., Serteyn, D., and Mouithys-Mickalad, A. Antioxidant and antiradical activities of Manihot esculenta Crantz (Euphorbiaceae) leaves and other selected tropical green vegetables investigated on lipoperoxidation and phorbol-12-myristate-13-acetate (PMA) activated monocytes. Nutrients, vol. 3, pp. 818-838, 2011.
[11] Banu, K. S., and Cathrine, L. General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science, vol. 2, pp. 25-32, 2015.
[12] Doughari, J. H. Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In Phytochemicals-A global perspective of their role in nutrition and health. (DV ed.). Nigeria, InTechOpen, 2012.
[13] Liu, R. H. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of nutrition, vol. 134, pp. 3479S-3485S, 2004.
[14] Mattson, M. P., and Cheng, A. Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses. Trends in neurosciences, vol. 29, pp. 632-639, 2006.
[15] Uddin, S. N. Antioxidant and antibacterial activities of Trema orientalis Linn: an indigenous medicinal plant of indian subcontinent. Oriental Pharmacy and Experimental Medicine, vol. 8, pp. 395-399, 2008.\
[16] Uddin, S. N., Akond, M. A., Mubassara, S., and Yesmin, M. N. Antioxidant and Antibacterial activities of Trema cannabina. Middle-East Journal of Scientific Research, vol. 3, pp. 105-108, 2008.
[17] Jayasri, M. A., Lazar, M., and Radha, A. A report on the antioxidant activity of leaves and rhizomes of Costus pictus D. Don. International Journal of Integrative Biology, vol. 5, pp. 20-26, 2009.
[18] Omoruyi, B. E., Afolayan, A. J., and Bradley, G. Chemical composition profiling and antifungal activity of the essential oil and plant extracts of Mesembryanthemum edule (L.) bolus leaves. African Journal of Traditional, Complementary and Alternative Medicines, vol. 11, pp. 19-30, 2014.
[19] Khan, M. S., Yusufzai, S. K., Kaun, L. P., Shah, M. D., and Idris, R. Chemical composition and antioxidant activity of essential oil of leaves and flowers of Alternanthera sessilis red from Sabah. Journal of Applied Pharmaceutical Science, vol. 6, pp. 157-161, 2016.
[20] Devi, O. Z., Rao, K. S., Bidalia, A., Wangkheirakpam, R., and Singh, O. M. GC-MS Analysis of Phytocomponents and Antifungal Activities of Zanthoxylum acanthopodium DC. Collected from Manipur, India. European Journal of Medicinal Plants, vol. 10, pp. 1-9, 2015.
[21] Ainsworth, E. A., and Gillespie, K. M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, vol. 2, pp. 875-877, 2007.
[22] Chang, C. C., Yang, M. H., Wen, H. M., and Chern, J. C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis, vol. 10, pp. 178-182, 2002.
[23] Ghazali, Q., and Yasin, N. H. M. The effect of organic solvent, temperature and mixing time on the production of oil from Moringa oleifera seeds. In IOP Conference Series: Earth and Environmental Science, vol. 36, pp. 1-7, 2016.
[24] Nwabueze, T. U., and Okocha, K. S. Extraction performances of polar and non-polar solvents on the physical and chemical indices of African breadfruit (Treculia africana) seed oil. African Journal of Food Science, vol. 2, pp. 119-125, 2008.
[25] Popa, O., Băbeanu, N. E., Popa, I., Niță, S., and Dinu-Pârvu, C. E. Methods for obtaining and determination of squalene from natural sources. BioMed research international, vol. 2015, pp. 1-16, 2015.
[26] Lakshmi, P. T. V., & Rajalakshmi, P. Identification of Phyto Components and Its Biological Activities of Aloe vera through the Gas Chromatography-Mass Spectrometry. International research journal of pharmacy, vol. 2, pp. 247-249, 2011.
[27] KhanYusufzai, S., Osman, H., Khan, M. S., Mohamad, S., Sulaiman, O., Parumasivam, T., Gansau, J. A., and Johansah, N. Design, characterization, in vitro antibacterial, antitubercular evaluation and structure–activity relationships of new hydrazinyl thiazolyl coumarin derivatives. Medicinal Chemistry Research, vol. 26, pp. 1139-1148, 2017.
[28] Rahman, F. S. A., Yusufzai, S. K., Osman, H., and Mohamad, D. Synthesis, Characterisation and Cytotoxicity Activity of Thiazole Substitution of Coumarin Derivatives (Characterisation of Coumarin Derivatives). Journal of Physical Science, vol. 27, pp. 77-87, 2016.
[29] Vázquez, L. H., Palazon, J., and Navaro-Ocaña, A. The pentacyclic triterpenes α, β-amyrins: A review of sources and biological activities. Phytochemicals, vol. 23, pp. 487-502, 2012.
[30] Fernie, A. R., Trethewey, R. N., Krotzky, A. J., and Willmitzer, L. Metabolite profiling: from diagnostics to systems biology. Nature reviews molecular cell biology, vol. 5, pp. 763-769, 2004.
[31] Likic, R., and Maxwell, S. R. Prevention of medication errors: teaching and training. British journal of clinical pharmacology, vol. 67, pp. 656-661, 2009.
[32] Di Marco, V. B., and Bombi, G. G. Mathematical functions for the representation of chromatographic peaks. Journal of Chromatography A, vol. 931, pp. 1-30, 2001.
[33] Perva-Uzunalić, A., Škerget, M., Knez, Ž., Weinreich, B., Otto, F., and Grüner, S. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. Food Chemistry, vol. 96, pp. 597-605, 2006.
[34] Scalbert, A., Johnson, I. T., and Saltmarsh, M. Polyphenols: antioxidants and beyond. The American journal of clinical nutrition, vol. 81, pp. 215S-217S, 2005.
[35] Edamatsu, H. I. D. E., Kawasaki, M. A. S. A. S. H. I., and Suga, N. O. B. U. O. Distribution of combination-sensitive neurons in the ventral fringe area of the auditory cortex of the mustached bat. Journal of neurophysiology, vol. 61, pp. 202-207, 1989.



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