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Comparative studies of phytochemical analysis and pharmacological activities of wild and micropropagated plant ethanol extracts of *Manihot esculenta*

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ABSTRACT

The present work was aimed to compare the phytochemical constitutes, antioxidant and cytotoxicity activities of wild and micropropagated *Manihot esculenta* ethanol (WMEE and MMEE) extracts. *M. esculenta* nodal regions were used for micropropagation. The explants were subjected to various chemicals for sterilization after that the explant inoculated on Murashige and Skoog (MS) medium supplemented with various concentration and combination of plant growth regulators. In micropropagation of *M. esculenta*, the plant growth regulators combination 6-benzyl amino purine (8.88 μM) along with naphthalene acidic acid (0.98 μM) and 1.16 μM kinetin (1.16 μM) were observed best shoot formation. Wild and micropropagated plants were extracted by ethanol which extracts performed the phytochemical quantitative screenings, FTIR and GCMS for identification of phytocompounds. The major secondary metabolites were presented in both sample extracts which are confirmed by preliminary phytochemical quantification tests. WMEE and MMEE extracts phytochemical constitutes were identified by FTIR and GC-MS analysis. In FTIR spectrum results, five and two major peaks were reported and 80 to 85 phytocompounds were identified by GCMS. Further, both sample extracts were studied the antioxidant property by various methods and colon carcinoma cell viability through MTT assay. In antioxidant activity, the WMEE and MMEE extracts showed significant activity in hydrogen peroxide scavenging. The cytotoxicity result the WMEE extract was potential in colon carcinoma cell than MMEE extract. From the phytochemical and pharmacological studies, the WMEE and MMEE extracts reported almost similar biological activities. So, the micropropagated plants will be used instead of wild plant for pharmaceutical purpose.

1. Introduction

M. esculenta Crantz, (Euphorbiaceae) commonly known as cassava and this species is one of the most important food crops in tropical, subtropics and mainly cultivated for its starching roots (Montagnac et al., 2009). In terms of production, it is the World's sixth most important crop and also over 500 million peoples uses this as staple food (Reilly et al., 2007). The tubers is the main source for the production of starch, monosodium glutamate, glucose and paper products. Tubers also used as feed for cattle.

Asia stands second among the cassava producing continents in the Word and around 13% of its production comes from India. This plant root tuber is used as food in many countries because the plant parts have high starch content with many nutrition properties (Mehran et al., 2014). Besides, cassava root and leaves parts are commonly used for the treatment of tumor, ringworm, sores and abscesses (Miladiyah, 2016). Cassava root is the main part whereas cassava leaves are readily available in considerable amounts as a by-product at the time of harvesting. This leaves have high protein content and used as animal feed. Furthermore, leaves have also been used against rheumatism, fever, headache, diarrhea, loss of appetite, hemorrhoid, inflammation (Okpuzor and Oloyede, 2009), microbial infection (Salami and Popoola, 2007).

The leaves of cassava contain crude protein ranging from 16.7 to 39.9%. They are rich in minerals such as iron, zinc, calcium, carbohydrates, manganese, magnesium and vitamins (Adewusi and Bradbury,

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Abbreviations

MS	Murashige and Skoog
FTIR	Fourier-transform infrared spectroscopy
GCMS	Gas Chromatogram Mass Spectroscopy
MTT	4, 5–dimethyl thiazol–2–yl)–5–Diphenyl tetrazolium bromide
BAP	6-benzyl amino purine
NAA	naphthalene acidic acid
KIN	kinetin
µM	micro molar

1993; Wobeto et al., 2006). In addition the essential amino acid profile of leaves of cassava is more than the FAO recommended reference protein intake and equal to that of soybean protein (Montagnac et al., 2009). Medicinal property rich cassava leaves can be made available throughout the year by plant tissue culture technique. This method is a tool for mass multiplication, germplasm conservations and secondary metabolites production (Dakah et al., 2014; Ozel et al., 2015; Palacio et al., 2012) and also help to produce disease free plantlets. So, the present work aimed to analyze phytochemical constitutes by FTIR, GCMS and pharmacological studies of wild and micropropagated plants ethanol extracts of *M. esculenta*.

2. Materials and methods

2.1. Chemicals

Bavistin-methyl-3-benzimidizole carbonate, mercuric chloride, sterile surgical blade, BAP, TDZ, KIN, NAA, 0.8% agar, 3% sucrose, ethanol, MTT (3-(4, 5–dimethyl thiazol–2–yl)–5–Diphenyl tetrazolium bromide), FCR (Folin-ciocalteu) reagent, trypsin, DMSO (Dimethyl Sulfoxide) and propanol were used for this study.

2.2. Indirect micropropagation

2.2.1. Explants selection and way of sterilization

M. esculenta nodal region were collected from field and used as explant for micropropagation. This nodal parts were cut into 0.4–0.6 cm length then treated with teepol for removing unwanted dusts on explants surface and washed with tap water twice. 10% of Bavistin (w/v) (methyl-3-benzimidizole carbonate) solution and rifampicin were used for explant sterilization to remove fungal and bacterial contamination respectively. Followed this, explants were subjected to 70 percentage ethanol and 0.12% (w/v) mercuric chloride solution and washed with sterile double distilled water. The sterile explant surface region was trimmed gently by sterile surgical blade and inoculated on to pre-cooled autoclaved medium.

2.2.2. Culture media and plant growth hormones for shoot and root formation

MS (Murashige and Skoog, 1962) full strength basal medium with various combinations and concentration of cytokinins and auxins were used for micropropagation of *M. esculenta*. The plant growth regulators like BAP (2.22–8.88 µM) alone or BAP along with KIN (1.16 µM) or TDZ (1.13 µM) or NAA (0.98 µM) were employed for this study. The sterile explants were inoculated on culture medium and every 15–20 days interval the media was changed for shoot multiplication and root formation.

2.3. Plant extracts preparation

The wild plant leaves and micropropagated plants were used for extraction. Both samples were dried at room temperature then grounded using mortar and pestle. 50 g of both wild plant leaves and micropropagated plants powder were extracted using 500 ml of ethanol by the method of cold extraction. After extraction, the solvent was removed through rotary evaporator and stored at 4 °C for further studies.

2.4. FTIR analysis

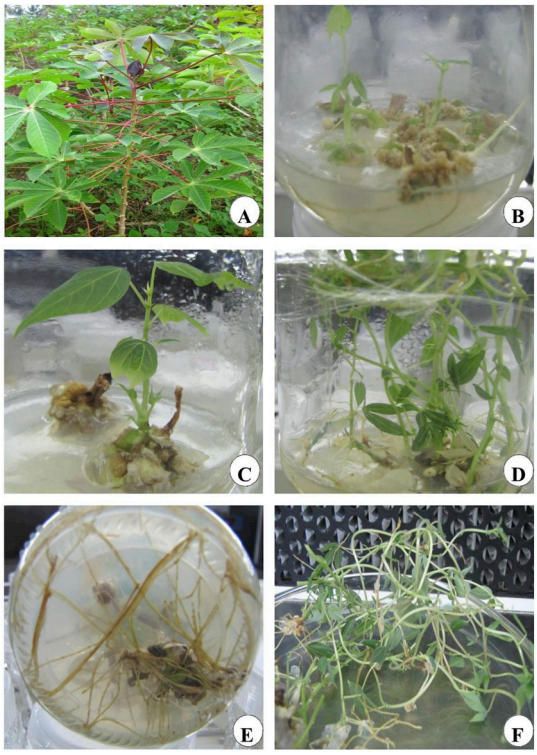
All the spectra were recorded with using BRUKER ALPHA 8400S FTIR spectrophotometer. Translucent sample discs were prepared by using dried WMEE and MMEE extracts encapsulated with KBr pellet. The sample was applied directly on the germanium piece of the infrared spectrometer with constant pressure. At the wavelength range between 3500/cm to 500/cm the absorbance data was collected. The FTIR spectrum of all samples was analyzed on the basis of peak values in the region of infrared radiation.

2.5. GCMS analysis

MWEE and MMEE extracts were examined through GC–MS analysis to identify the phytocompounds. 1 µl of WMEE and MMEE extracts were injected into the GC PerkinElmer model instrument. The temperature was 260 °C in the initial stage and increased to 300 °C for 6 min and the oven temperature was 60 °C for 2 min. The temperature was 240 °C in mass detector conditions and also for ion source. The ionization mode electron impact at 70 eV, a scan time 0.2 s and scan interval of 0.1 s (Anupama et al., 2014). TurboMass ver 5.4.2 software

Table 1
Effect of MS medium and different concentration and combination of plant growth regulators on initiation, multiple shoot formation from nodal explant of *M. esculenta*.

Plant growth hormones (µM)	Callus percentage	Mean number of shoot/explant	Mean number of shoot/by shoot subculture	Mean shoot length (cm)
BAP (2.22)	23.18	1.33 ± 0.57^{c}	4.33 ± 0.57^{b}	4.07 ± 0.04^{d}
BAP (4.44)	28.44	2.0 ± 0.0^{b}	6.0 ± 1.0^{ab}	4.27 ± 0.05^{c}
BAP (6.66)	35.17	2.3 ± 0.57^{b}	6.33 ± 1.5^{ab}	4.53 ± 0.09^{b}
BAP (8.88)	41.20	3.33 ± 0.57^{a}	7.0 ± 1.0^{a}	5.07 ± 0.03^{a}
BAP (2.22) + TDZ (1.13)	26.51	2.0 ± 0.0^{c}	4.0 ± 1.0^{c}	4.21 ± 0.36^{d}
BAP (4.44) + TDZ (1.13)	31.82	2.33 ± 0.57^{bc}	4.33 ± 0.57^{c}	4.73 ± 0.08^{c}
BAP (6.66) + TDZ (1.13)	37.74	3.0 ± 0.0^{ab}	6.0 ± 1.0^{b}	5.21 ± 0.06^{b}
BAP (8.88) + TDZ (1.13)	46.23	3.33 ± 0.57^{a}	8.33 ± 0.57^{a}	5.64 ± 0.07^{a}
BAP (2.22) + KIN (1.16)	25.11	2.0 ± 0.0^{c}	6.67 ± 0.57^{c}	4.14 ± 0.30^{d}
BAP (4.44) + KIN (1.16)	29.64	2.33 ± 0.57^{c}	8.67 ± 0.57^{b}	4.41 ± 0.07^{c}
BAP (6.66) + KIN (1.16)	38.26	3.33 ± 0.57^{b}	10.0 ± 1.0^{b}	5.57 ± 0.06^{b}
BAP (8.88) + KIN (1.16)	44.87	4.33 ± 0.57^{a}	12.0 ± 1.0^{a}	6.18 ± 0.04^{a}
BAP (2.22)+KIN (1.16)+NAA (0.98)	46.45	5.0 ± 1.0^{c}	13.0 ± 1.0^{c}	6.29 ± 0.02^{d}
BAP (4.44)+KIN (1.16)+NAA (0.98)	64.16	5.67 ± 0.57^{bc}	15.0 ± 1.0^{b}	6.78 ± 0.03^{c}
BAP (6.66)+KIN (1.16)+NAA (0.98)	72.37	7.0 ± 1.0^{ab}	16.67 ± 0.57^{ab}	7.12 ± 0.04^{b}
BAP (8.88)+KIN (1.16)+NAA (0.98)	84.10	8.33 ± 0.57^{a}	18.0 ± 1.0^{a}	8.22 ± 0.04^{a}



A – Habit; B – Shoot formation from callus; C & D – Shoot development; E – Rooting; F – Multiple shoot with root

Fig. 1. Indirect micropropagation of *Manihot esculenta*.

was used and the mass spectrums of compounds were interpreted by using database of National Institute Standard and Technology (NIST), library 2008.

2.6. Antioxidant assays

2.6.1. DPPH assay

DPPH scavenging analysis was performed in WMEE and MMEE extracts by the method of Szabo et al. (2007). 21 mg of the WMEE and MMEE extracts were weighed and dissolved in 1 ml DMSO separately to a solution of 21 mg/ml concentration and was diluted by serial dilution method. 200 µl of DPPH solution mixed with 10 µl of WMEE and MMEE extracts separately and incubated at 37 °C for 20 min. The absorbance was calculated by ELISA reader at 490 nm.

2.6.2. Nitric oxide assay

Twenty one mg of WMEE and MMEE extracts were separately dissolved in 2 ml of DMSO to get 21 mg/ml concentration, these solution were serially diluted with dimethyl sulfoxide to get lower concentration. The reaction mixture was prepared by 2 ml of 10 mM sodium nitroprusside with 0.5 ml phosphate buffer saline and 0.5 ml WMEE and MMEE extract separately. Further it was incubated at 25 °C for 90 min. After that, 1 ml of sulfanilic acid was poured and mixed well, then 1 ml of N-(1-Naphthyl) ethylenediamine was poured and allowed to stand for 30 min at normal temperature. The mixture was measured at 540 nm (Hazra et al., 2008; Sánchez-Moreno, 2002).

Table 3
Preliminary phytochemical screening of WMEE and MMEE extracts.

Secondary metabolites	Chemical test	WMEE	MMEE
Alkaloids	Dragendroff's test	+	+
	Mayer's test	–	–
	Wagner's test	+	+
	Hager's test	+	+
Flavonoids	10% HCl & 5% NaOH test	+	+
	Alkaline test	+	+
Tannins	5% $FeCl_3$ Test	+	+
Steroids	Libermann - Burchard's test	+	+
Triterpenoids	Libermann - Burchard's test	+	+
	Salkowski's test	+	+
Saponins	Foam Test	–	–
Glycosides	Killer & Kilian test	–	–
Gum & Mucilages	Whistle & BeMiller test	–	–
Fixed oils	Spot test	–	–
Anthraquinones	NH_4OH test	–	–

+ indicates presence; - indicates absence.

2.6.3. Scavenging of hydrogen peroxide radicals

Hydrogen peroxide radical scavenging ability of WMEE and MMEE extracts were evaluated by Jayaprakasha et al. (2004) method. Thirty milligram of each extracts were weighted and separately dissolved in 10 ml of methanol. This solution was serially diluted with methanol to obtain the lower dilutions. Seven different concentrations of 1 ml of the extracts were added to 2 ml of H_2O_2 solution. At 230 nm absorbance was measured.

2.6.4. Hydrogen radical scavenging activity by P-nitrosodimethyl aniline

Thirty milligram of each samples were weighted and dissolved separately in 5 ml of DMSO. The solutions were diluted with DMSO to obtain lower dilution. Ferric chloride, EDTA, ascorbic acid (0.1 mM, 0.5 ml each), H_2O_2 (2 mM, 0.5 ml), and pNDA (0.01 mM, 0.5 ml) in phosphate buffer pH 7.4 (20 mM) were added with various concentrations of extracts (0.5 ml) and made a final volume of 3 ml. 0.5 ml sample and 2.5 ml of phosphate buffer (pH 7.4) was used as blank. Absorbance was measured at 440 nm.

2.7. In vitro cytotoxicity activity

The *in vitro* cytotoxicity activity of WMEE and MMEE were carried out by MTT assay according to the method described by Denizot and Lang (1986). The micro plate wells were filled with 100 µl DMEM growth medium along with cytotoxicity cells. The plates were kept for

Table 2
Effect of different concentration and combination of growth hormones in MS media on rooting of *M. esculenta*.

Plant growth hormones (µM)	Mean number shoots	Mean number of roots	Mean number root length (cm)
BAP (2.22) + NAA (0.98)	9.0 ± 1.0^{d}	7.67 ± 1.52^{ab}	4.56 ± 0.25^{a}
BAP (4.44) + NAA (0.98)	11.33 ± 0.57^{c}	8.67 ± 1.52^{a}	4.30 ± 0.20^{a}
BAP (6.66) + NAA (0.98)	13.0 ± 1.0^{b}	5.67 ± 0.57^{b}	3.60 ± 0.25^{b}
BAP (8.88) + NAA (0.98)	17.67 ± 0.57^{a}	6.0 ± 1.0^{b}	3.10 ± 0.1^{d}
BAP (2.22) + KIN (1.16) + NAA (0.98)	10.33 ± 0.57^{d}	11.0 ± 1.0^{a}	5.36 ± 0.15^{a}
BAP (4.44) + KIN (1.16) + NAA (0.98)	14.0 ± 1.0^{c}	9.33 ± 1.52^{a}	5.0 ± 0.15^{b}
BAP (6.66) + KIN (1.16) + NAA (0.98)	18.0 ± 1.0^{b}	6.33 ± 0.57^{b}	3.60 ± 0.10^{c}
BAP (8.88) + KIN (1.16) + NAA (0.98)	22.0 ± 1.0^{a}	5.33 ± 1.52^{b}	3.30 ± 0.20^{d}

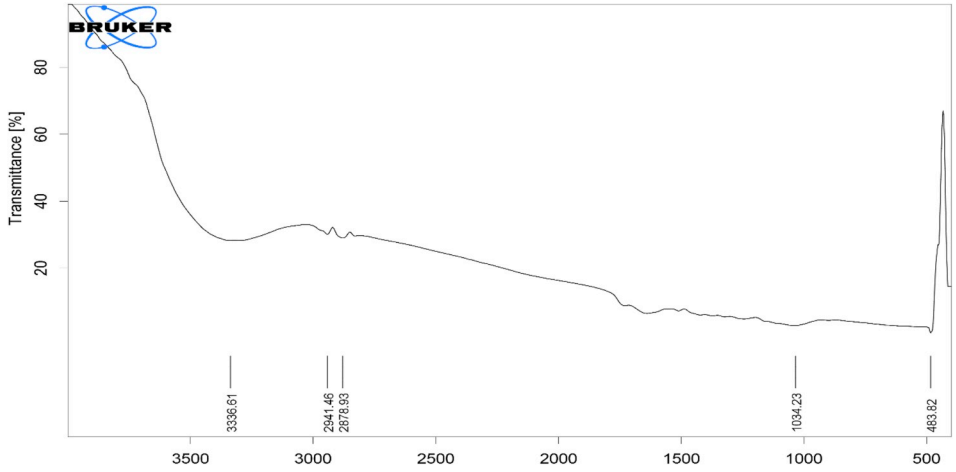


Fig. 2. FTIR spectra analysis of WMEE extract.

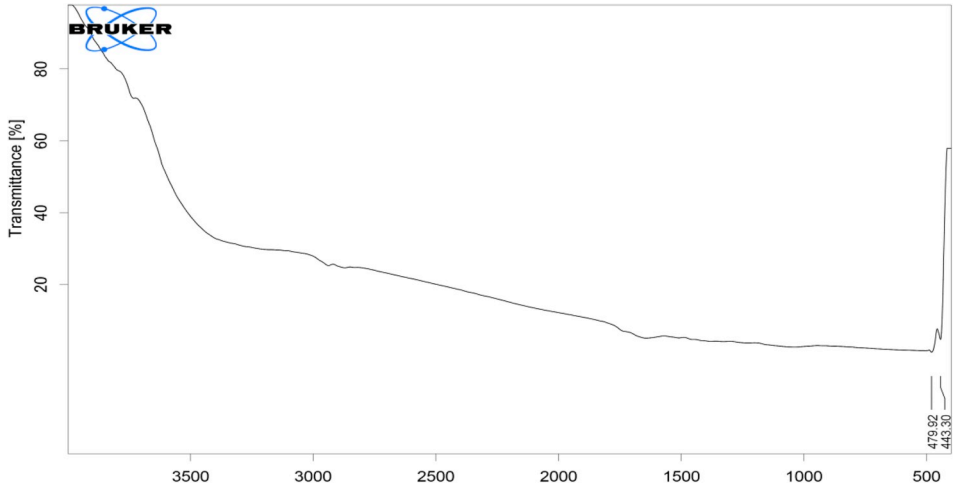


Fig. 3. FTIR spectra analysis of MMEE extract.

Table 4
IR spectral peak values and functional groups obtained from WMEE and MMEE extracts.

Sample	Frequency (cm^{-1})	Intensity	Assignment	Characterization
WMEE extract	3336.61	Strong, broad	–OH group	Alcohols, phenols
	2941.46	Medium	C–H group	Alkanes
	2878.93	Medium	C–H group	Alkanes
	1034.23	Strong	C = O stretch	Aldehydes, saturated aliphatic
	483.82	Medium	-C=C	Alkenes
MMEE extract	497.92	Medium	C–H groups	Alkanes
	443.30	Medium	C–H groups	Alkanes

24 h at 37 °C under 5% CO_2 in a humidified atmosphere. Then, the medium was replaced with fresh growth medium and different concentrations of WMEE or MMEE extracts. After 48 h of incubation, the medium was removed and 20 µl MTT reagents were added. After incubation for 4 h, the MTT reagent was removed before adding 100 µl DMSO to each well and gently shaken. The absorbance was then determined by ELISA reader at 492 nm. Control wells received only the media without the tested samples. Inhibition of cell growth by the test extracts was calculated as % cytotoxicity activity as follows, % cytotoxicity activity = $(A_0 - A_1/A_0)$ x 100, where A_0 and A_1 refer to the absorbance of control and the sample, respectively.

3. Results

3.1. Indirect micropropagation of M. esculenta

Callus was originated from 24th days of nodal culture in MS medium augmented with various combination and concentration of cytokinin and auxins (Table 1). Among the four different types plant growth regulators (PGR) combination and concentration, BAP (8.88 µM) and KIN (1.16 µM) and NAA (0.98 µM) combination produced highest percentage of callus as well as shoot number and shoots length (Table 1). Callus production percentage 84.10 along with mean number of shoots 8.33 ± 0.57 (Fig. 1b), mean number of shoots in sub culture 18.0 ± 1.0 (Fig. 1c) and shoot length 8.22 ± 0.04 (Fig. 1c) was observed. The other PGR combination like BAP alone or BAP with TDZ and BAP with KIN also produced callus and shoots in higher concentration, but not well than BAP (8.88 µM) along KIN (1.16 µM) and NAA (0.98 µM) combination. Different concentration of the same PGR combination produced dominant callus and shoot formation than other combination of PGR (Table 1).

3.1.1. Root formation

Well-developed first subculture shoots were transferred to two different PGR combinations for root initiation. Among the two combination of PGR, the combination of BAP (2.22 µM) + KIN (1.16 µM) and NAA (0.98 µM) were initiated highest number of root formation and root length (Fig. 1d). Table 2 showed the mean number of root

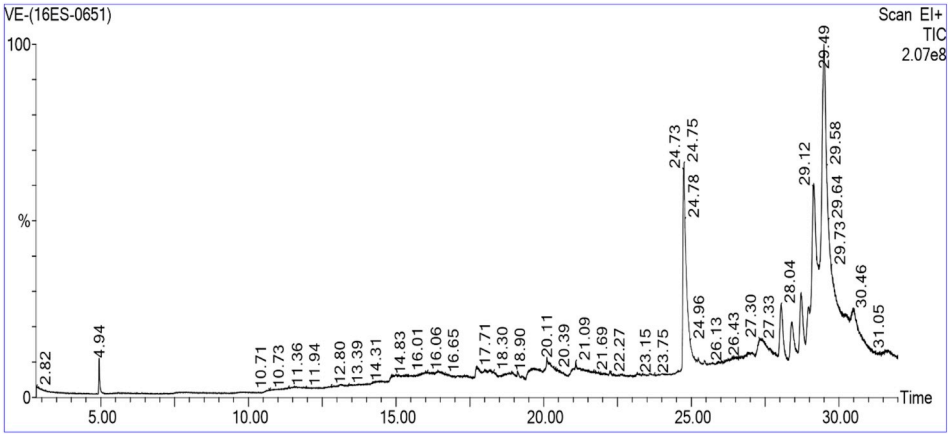


Fig. 4. Gas chromatogram of phytochemical constitute of WMEE extract.

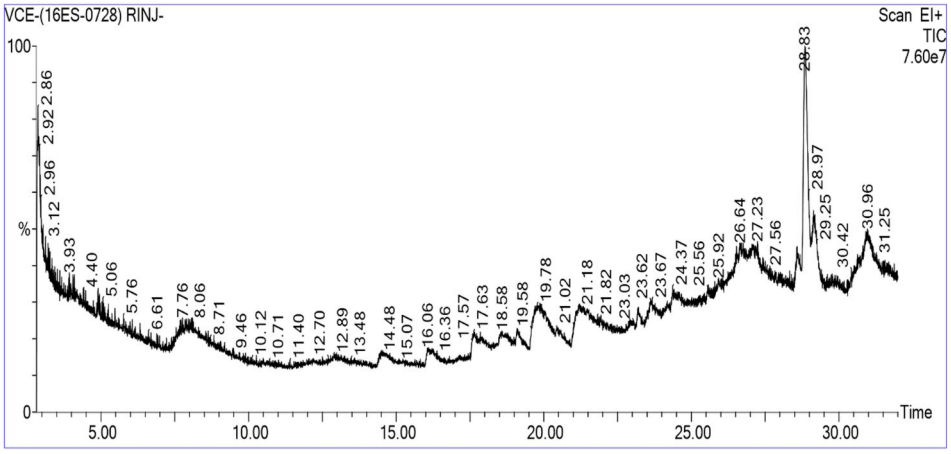


Fig. 5. Gas chromatogram of phytochemical constitute of MMEE extract.

11.0 ± 1.0 and mean root length 5.36 ± 0.15 from this medium combination but less number shoots were formed by this PGR combination compared to higher concentration (Table 2). Another PGR combination was also produced significant number of roots but not better than BAP (2.22 μM) + KIN (1.16 μM) and NAA (0.98 μM) combination.

3.2. Phytochemical study

The qualitative screening of secondary metabolites was analyzed in WMEE and MMEE extracts. Table 3 shows the presence and absence of secondary metabolites and the test confirmed that the presence of similar number secondary metabolites in both the samples. WMEE and MMEE extracts were positive in alkaloids, flavonoids, tannins, steroids and triterpenoids. Likewise saponins, glycosides gum & mucilages, fixed oils and anthraquinones were absent in both sample extracts.

3.3. FTIR analysis

The results of FTIR spectra of WMEE and MMEE extracts were analyzed from 3500 to 500 cm^{-1}. The spectra were given in Figs. 2 and 3 and the peak intensity, assignment, characters were reported in Table 4. The absorption spectra of WMEE extract have five strong, sharp, medium peaks which are shown in Fig. 2. The dominant band was observed at 3336.61, 2941.46, 2878.93, 1034.23 and 483.82 cm^{-1}. The band at 3336.61 was due to alcohols and phenolic compounds which assignment is –OH stretch. Three peaks such as 2941.46, 2878.93 and 483.82 were reported as alkanes group and that peaks are C–H group and –C═C assignments with medium intensity. Another one peak 1034.23 was aldehydes, saturated aliphatic group compound with strong intensity peak. Two medium alkane group peaks (497.92 and 443.30) were identified from MMEE extract and this peaks stretch was C–N.

3.4. GC-MS analysis

The WMEE and MMEE extracts were subjected to GC-MS for identification of the phytochemical constitutes. Figs. 4 and 5 were the chromatogram of WMEE and MMEE extracts and seventy nine and eighty four phytocompounds were identified from these extracts. In both samples phytocompounds were present between retention times 2 to 30. In WMEE extract four bioactive compounds were identified and this bioactive compounds name, molecular formula, molecular weight, Chemical Abstracts Service (CAS) number and its bioactive uses were tabulated in Table 5. The bioactive compounds are 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E), squalene, vitamin E and lupeol. Commonly this bioactive compounds are reported as antimicrobial, anti-inflammatory, anticancer, activities, antioxidant, antitumor, chemo preventive, lipoxygenase-inhibitor and pesticide.

Eight bioactive compounds were identified in eighty four phytocompounds of MMEE extract (Fig. 5) and its name, molecular weight, formula, CAS number and bioactive uses were given in Table 6. The bioactive compounds are N-hexadecanoic acid with antioxidant, nematicide, pesticide, lubricant properties, tridecanoic acid is used in bacterial and fungal infections, octadecanoic acid is used for inflammation, pentadecanoic acid have antioxidant property, dodecanoic acid is used in

Table 5
Bioactivities of phytocomponents identified in the WMEE extract by GC-MS.

Compound name	Mol. Formula	Mol. Weight	CAS number	Bioactive uses
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)	$C_{30}H_{50}$	410	111-02-4	Antimicrobial, anti-inflammatory activities (Sivakumar and Gayathri, 2015).
Squalene	$C_{30}H_{50}$	410	7683-64-9	Antibacterial, antioxidant, antitumor, cancer preventive, chemo preventive, lipoxygenase-inhibitor and pesticide (Agnel Ruba and Mohan, 2014).
Vitamin E	$C_{29}H_{50}O_2$	430	59-02-9	Antioxidant activity (Voljč et al., 2011)
Lupeol	$C_{30}H_{50}O$	426	545-47-1	Anticancer, anti-inflammatory and antioxidant activities (Maruthupandian and Mohan, 2011).

Table 6
Bioactivities of phytocomponents identified in the MMEE extract by GC-MS.

Compound Name	Mole. Formula	Mole. Weight	CAS number	Bioactive uses
N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	57-10-3	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors (Jegadeeswari et al., 2012; Upgade and Bhaskar, 2013).
Tridecanoic acid	$C_{13}H_{26}O_2$	214	638-53-9	Antibacterial and antifungal activities (Chandrasekaran et al., 2011).
Octadecanoic acid	$C_{18}H_{36}O_2$	284	57-11-4	Anti-inflammatory (Aparna et al., 2012; Rajeswari et al., 2012).
Pentadecanoic acid	$C_{15}H_{30}O_2$	242	1002-84-2	Antioxidant activity (Elezabeth and Arumugam, 2014).
Dodecanoic acid	$C_{12}H_{24}O_2$	200	143-07-7	Antibacterial, antiviral and antifungal activities (Özçelik et al., 2005).
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	544-63-8	Antioxidant, cancer-preventive, hypercholesterolemic and cosmetic (Zayed et al., 2014).
Cyclotrisiloxane, Hexamethyl	$C_6H_{18}O_3Si_3$	222	541-05-9	Antioxidant activity (Prakash and Suneetha, 2014)
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl	$C_{16}H_{50}O_7Si_8$	578	19095-24-0	Antimicrobial activity (Kumaradevan et al., 2015)

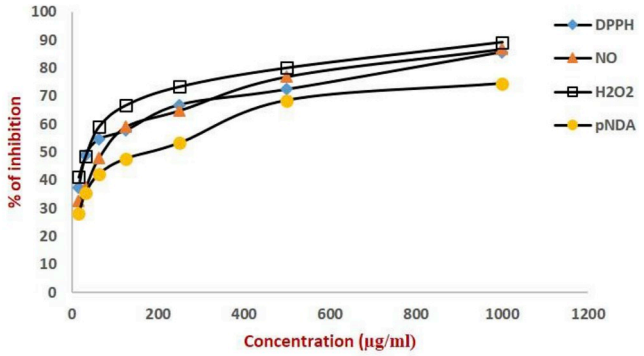


Fig. 6. Various antioxidant activities of WMEE extract.

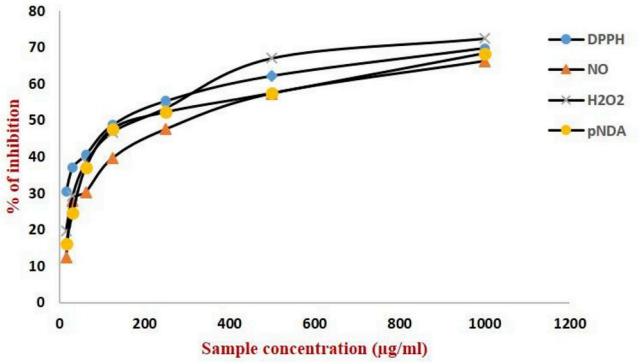


Fig. 7. Various antioxidant activities of MMEE extract.

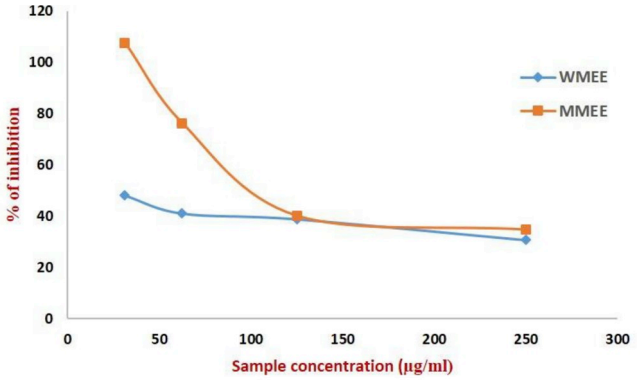


Fig. 8. Cytotoxicity activities of WMEE and MMEE extracts.

bacterial, viral and fungal infections, tetradecanoic acid is used in oxidant stress and cosmetic, cyclotrisiloxane hexamethyl work on oxidant stress and octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl have antimicrobial activity.

3.5. Antioxidant activity

The four different antioxidant assays were carried out in WMEE and MMEE extracts and the results were shown in Figs. 6 and 7. The maximum percentage of free radical inhibition was observed at higher concentration (1000 µg/ml) of both samples. In WMEE extract, hydrogen peroxide has shown the maximum inhibition at each concentration than other antioxidant assays. The highest percentage inhibition of each assays were 89.11, 86.8, 85.57 and 74.38 noticed in hydrogen peroxide, nitric oxide, DPPH and pNDA assay respectively. Hydrogen peroxide has exhibited maximum percentage of inhibition than other antioxidant assay in MMEE extract and highest inhibition percentage 72.4 was noticed at 1000 µg/ml concentration (Fig. 7). In DPPH, pNDA and nitric oxide antioxidant activities were observed at 1000 µg/ml concentration and the percentage of inhibitions were 69.7, 68.3 and 66.2 respectively.

3.6. Cytotoxicity activity

The *in vitro* cytotoxicity activities of WMEE and MMEE were examined against human colon carcinoma cell line (HT-29 cell line) by MTT assay. Fig. 8 showed cell viability percentage of HT-29 cells and percent of cell inhibition was observed in all the sample with concentration dependent manner. The minimum percentage of cell viability was observed at 250 µg/ml concentration of both samples and the percentage of cell viabilities were 34.82 and 30.77. When increase in the sample concentration the percentage of cell viability decreases and highest percentage of cell viabilities of both samples were 48.11 and 107.75. The IC_{50} values of these extracts are 23.77 µg/ml, 133.45 µg/ml, respectively.

4. Discussion

Plant tissue culture is the most efficient technique for secondary metabolites production and these secondary metabolites were used in pharmacological products, food additives and agrochemicals. Many literature were available for phytochemical analysis and its biological activities of plants like *Scrophularia kakudensis* (Manivannan et al., 2016), *Ceropegia juncea* (Saraswathy et al., 2017) and *Baccharoides anthelmintica* (Kalimuthu et al., 2016). The present study was aimed to analyze phytochemical constitutes and biological activities of WMEE and MMEE extracts. In micropropagation of *M. esculenta*, BAP, KIN and NAA at the level of 8.88, 1.16 and 0.98 µM respectively were found to initiate a maximum of 8.33 mean shoots in initiation and 18 mean shoots in subculture. The action of BAP in bud breaking has already been reported for many Euphorbiaceae plants such as *Ricinus communis* (Lakshmi and Bahadur, 1997), *Cleistanthus collinus* (Quraishi et al., 1996) and *Euphorbia officinalis* (Verma and Kant, 1999). Multiple shoot formation was observed mostly in the presence of high concentration of cytokinins and low concentration of auxin or cytokinin in *M. esculenta* (Carretero et al., 2007; Le et al., 2007) and *Cleistanthus collinus* (Quraishi et al., 1996). In the present study BAP in combination with KIN and NAA increased the number of shoots. It is in accordance with the result obtained in *M. esculenta* by Mapayi et al. (2013) and *Embilica officinalis* by Verma and Kant (1999). Experiments carried out with reference to rooting of micro-shoots, in most of the Euphorbiaceae members MS medium supplemented with auxin(s) favored the rhizogenesis (Jyoti et al., 2000). The improvement of overall quality and number of roots was observed only in full strength medium in most of the members of Euphorbiaceae. Similar trend of results were observed in the present study for *M. esculenta.* Again it was known that NAA was found to be more suitable for the root induction with BAP and KIN. The effectiveness of NAA in rooting has been reported for Euphorbiaceae plants like *Cleistanthus collins* (Quraishi et al., 1996) and *Emblica officinalis* (Verma and Kant, 1999). MS medium supplemented with NAA or IBA induced callus at the base in *Cleistanthus Collins* (Acharya and Shrivastava, 2008). However, in the present study it was observed that the roots were formed without the intervention of callus on MS medium containing NAA along with BAP and KIN.

The medicinal and pharmacological actions of plant are often dependent on the presence of the secondary metabolites (Heinrich et al., 2017). By the preliminary phytochemical analysis, WMEE and MMEE extracts possess same secondary metabolites such as alkaloids, flavonoids, tannins, steroids and triterpenoids. Alkaloids are naturally occurring nitrogenous organic compounds with antimicrobial properties (Kasolo et al., 2010). Flavonoids and tannins are major group of phenolic compounds that act as an antioxidants, anti-inflammatory properties, anticarcinogenic and antimutagenic activities (Kasolo et al., 2010). Triterpenoids are used for pain relieving, antipyresis,

hepatoprotective, soothing and antidiabetic properties (Ovensná et al., 2004).

Gas chromatogram is a best tool for separation of organic compounds and these compounds are identified by mass spectroscopy. In current research, plant derived single compound of crude extracts are identified with compound name, molecular weight and molecular formula through GCMS analysis (Fernie et al., 2004; Robertson, 2005; Sharma and Vijayvergia, 2015). In the present study, seventy nine and eighty four phytocompounds were identified from WMEE and MMEE extracts by GCMS. Four bioactive compounds were identified in WMEE extract. The bioactive compounds are 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E), squalene, vitamin E and lupeol. These bioactive compounds commonly have antimicrobial, anti-inflammatory (Maruthupandian and Mohan, 2011; Sivakumar and Gayathri, 2015), antioxidant (Voljč et al., 2011), antitumor, cancer preventive, pesticide activities (Agnel Ruba and Mohan, 2014). Likewise in MMEE extract, eight known bioactive compounds were identified and they are N-hexadecanoic acid, tridecanoic acid, octadecanoic acid, pentadecanoic acid, dodecanoic acid, tetradecanoic acid, cyclotrisiloxane hexamethyl and octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl with antioxidant (Elezabeth and Arumugam, 2014; Prakash and Suneetha, 2014), hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors, antibacterial, antifungal activities, anti-inflammatory, antiviral activity, cancer-preventive, hypercholesterolemic and cosmetic bioactivity uses (Aparna et al., 2012; Özçelik et al., 2005; Rajeswari et al., 2012). So present analysis is believed to help in the identifying antioxidant and cytotoxicity activities of WMEE and MMEE extracts.

IR spectrum provided essential information on the molecular structure of organic and inorganic components, and one of the most multifaceted analytical techniques for the non-destructive, chemical characterization of samples (D'Angelo and Zodrow, 2011; Lis et al., 2005; Rossman, 2006; von Aulock et al., 2014). IR spectrum analysis of WMEE and MMEE extracts exhibited possibility of identifying effective functional groups in the chemical constituents and possible whereby identify the different compounds, since each compound has own fingerprint, and can be distinguished between aromatic and non-aromatic compounds and alcohol, phenol, aldehydes, saturated aliphatic and alkenes. Five and two peaks were identified in WMEE and MMEE extracts and these peaks are commonly have alcohols, phenolic, aldehydes, saturated aliphatic and alkane group compounds.

Oxidation stress, a key player in several diseases such as cancer, diabetic, aging, inflammatory diseases etc. Imbalance between formation and neutralization of peroxidation leads to oxidative stress. Many chemo and herbal drugs are used for controlling oxidative stress. Herbal remedies may react as pro-oxidant related on the concentration and on the environment redox conditions. Therefore, the present study analyzed the various antioxidant activities of WMEE and MMEE extracts. Cassava leaves containing high phytochemical constituents and which act against many diseases such as rheumatism, fever, headache, diarrhea, hemorrhoid, inflammatory (Okpuzor and Oloyede, 2009) and microbial activity (Salami and Popoola, 2007). In addition, this species leaves have flavonoid compound rutin, which have highly antioxidant activity. From the antioxidant results, both sample extracts were showed maximum percentage of inhibition. The percentage inhibition of WMEE extract was 89.11 (hydrogen peroxide), 86.8 (nitric oxide), 85.57 (DPPH), 74.38 (pNDA) and MMEE extract percentage of inhibition was 72.4 (hydrogen peroxide), 69.7 (DPPH), 68.3 (pNDA), 66.2 (nitric oxide).

The changes in cellular hemostasis, that results in number of changes in the adaptive immunity as well as their survival, multiplication and metabolic activity is cytotoxicity (Todryk et al., 2001). The cytotoxicity of HT-29 cell result reported minimum percentage of cell viability at 250 µg/ml concentration and the minimum percentage of cell viability was 4.82 and 30.77 from WMEE and MMEE extracts respectively. Squalene is a triterpenoid compound and used as antioxidant, antitumor and cancer preventive (Agnel Ruba and Mohan, 2014). This squalene was present in WMEE extracts and this compound maybe the reason for cytotoxicity activity of this study. Likewise, in MMEE extract have tetradecanoic acid compound and this is used as antioxidant, cancer-preventive (Zayed et al., 2014) and the presence of this compound maybe the reason for antioxidant and cancer preventive activities of this plant.

5. Conclusion

From this study, micropropagated plant have number of phytocompounds with some bioactive compounds. The bioactive compounds are used for various diseases such as oxidative stress, inflammation, microbial infection and cancer. So, the bioactive compounds will be produced large amount through micropropagation. In addition, significant results of antioxidant and cytotoxicity activities were reported by this study and the results were almost similar in both samples. For confirmation of antioxidant and colon cancer activities through animal model, the micropropagated plants will be used instead of wild plants.

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