Cardioprotective Potential of the Ethanol and Water Extracts of Four Psilocybin Mushrooms on Angiotensin II-Induced Hypertrophy and Oxidative Stress on H9C2 Cardiomyocytes

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Article

Cardioprotective Potential of the Ethanol and Water Extracts of Four Psilocybin Mushrooms on Angiotensin II-Induced Hypertrophy and Oxidative Stress on H9C2 Cardiomyocytes

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Abstract: Psilocybin-containing mushrooms, commonly known as magic mushrooms have antidepressant effect, however, their safety in cardiovascular diseases such as heart failure is not fully known and needs to be investigated. Cardiac hypertrophy is an independent risk factor for heart failure morbidity and mortality. Angiotensin II (Ang-II) plays a major role in the pathogenesis of cardiac hypertrophy. We investigated the cardiovascular safety of extracts of *Panaeolus cyanescens, Psilocybe natalensis, Psilocybe cubensis*, and *Psilocybe cubensis leucistic A+ strain* mushrooms, well-known psilocybin-containing mushrooms in the *Panaeolus* and *Psilocybe* genus on Ang II-induced hypertrophy oxidative stress. The four mushrooms were grown, dried and extracted with 70% ethanol, cold and hot water. Extracts were tested for cytotoxicity on H9C2 cardiomyoblast cells. The cardiomyocytes were induced with (10 μM) AngII and treated with the three extracts of the four mushrooms over 48 hours. Control cells were serum starved but neither AngII induced nor treated while AngII cells were serum starved and stimulated with AngII but not treated. Losartan, an inhibitor of AngII type 1 receptor was used as positive control. Effects of the extracts on actin-filament labelling and cell surface area, mitochondrial activity, reactive oxygen species (ROS) and atrial natriuretic peptide levels were determined. Stimulation with AngII lowered cell viability, increased the cell width measurements and intracellular ROS levels significantly compared to control cells. The results indicated that the ethanol and water extracts of the four psilocybin mushrooms did not exacerbate the angiotensin II-induced hypertrophy conditions, but the extracts had cardio-protective activity against angiotensin II-induced oxidative stress. The phytochemical analysis of the extracts confirmed detections of known compounds with antioxidant and anti-inflammatory effects in the water and ethanol extracts of these four psilocybin mushrooms.

Keywords: reactive oxygen species; angiotensin II; hypertrophy; psilocybin mushrooms; heart failure

1. Introduction

Depression is a burden to society and associated with chronic stress and aging [1]. Psilocybin-containing mushrooms have been used by different tribes to improve quality of life and for mind healing [2]. Many studies have also demonstrated the antidepressant effects of psilocybin (4-phospholoxy-N-N-dimethyltryptamine), the classic psychedelic agent occurring naturally in psilocybin-containing mushrooms [3,4]. Consequently, the use and awareness of psilocybin-containing mushrooms, commonly known as magic mushrooms is increasing. However, psilocybin and psilocybin mushrooms also lead to a temporary increase in heart rate and blood pressure which may pose as risk especially for users suffering from cardiovascular diseases [5]. Since depression is

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associated with aging people that are prone to cardiovascular disease such as hypertension and heart failure, investigating safety of the mushroom usage in these conditions is crucial.

Heart failure is an international public health problem of pandemic proportions and studies showed that about 64.3 million people globally are living with a heart failure condition [6,7]. Cardiomyocyte hypertrophy which is a major consequence of pressure and/or volume overload is considered a significant diagnostic component and plays a key role in the progression of heart failure [8]. Cell enlargement and apoptotic loss of cardiomyocytes are key pathological changes in cardiac hypertrophy [9,10]. Many factors are involved in the pathogenesis and regulation of cardiomyocyte hypertrophy including angiotensin II (AngII). Angiotensin II is a key factor of the renin-angiotensin system that induces cell hypertrophy, differentiation and apoptosis through activation of various intracellular signalling molecules including calcineurin, mitogen-activated protein kinase and many other factors [11].

Angiotensin II has two receptors, AngII type 1 (AT1R) which is known to mediate pro-hypertrophic effects of AngII and AngII type 2 (AT2R) that attenuates the AT1R activation-induced hypertrophy [12]. A fibroblast-derived factor is identified as a biochemical process used by AngII to stimulate direct cardiomyocyte hypertrophy which can be blocked by using bromodeoxyuridine, a fibroblast proliferation inhibitor [12]. Many studies also showed that AngII stimulated protein synthesis which could also be eliminated by losartan, the AT1R blocker, further indicating direct role of AngII in the production of some fibroblasts factor [12,13]. Furthermore, studies have also showed that the pro-hypertrophic effects of AngII are also mediated through mitochondrial and induced-cell death by activating NAD(P)H oxidase through ATR1 receptors leading to increased generation of reactive oxygen species (ROS) and oxidative stress [14,15]. Oxidative stress is the lack of balance state where production of ROS such as superoxide, hydrogen peroxide and hydroxyl radicals exceed the antioxidant defences [13].

Plasma levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), well-known as hall markers of heart failure, have been found to increase with the severity of heart failure [16]. Their main physiological effects are to suppress progression of heart failure by inducing several effects that includes inhibition of the renin-angiotensin-aldosterone and promoting vasodilation and natriuresis [16].

This study aimed at investigating for the first time the risks and/or safety of *Panaeolus cyanescens*, *Psilocybe natalensis*, *Psilocybe cubensis* and *Psilocybe cubensis leucistic A+ strain* mushrooms, well-known psilocybin-containing mushroom in the genus *Panaeolus* and *Psilocybe*, on AngII-induced hypertrophy using a rat H9C2 cardiomyoblast cells model which is well-known and a widely used in vitro cell model with accepted reliability in cardiovascular drug discovery [17].

2. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

2.1. Cytotoxicity of the Extractsn

The cytotoxicity results of the extracts on H9C2 cardiomyocytes showed that the three extracts of the four magic mushrooms were not toxic when comparing their LC50 values with the positive control doxorubicin, a well-known toxic drug, Table 6.1.

Table 1. Cytotoxicity effects of the extracts over 48 hours on H9C2 cardiomyocytes.

Sample	LC50 (µg/mL)
Pan cyanescens cold-water (PC)	66.1 ± 1.8
Pan cyanescens hot-water (PH)	>100
Pan cyanescens 70% ethanol (PE)	> 100
P. cubensis cold-water (GC)	>100

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P. cubensis hot-water (GH)	>100
P. cubensis 70% ethanol (GE)	>100
P. A+ strain cold-water (AC)	>100
P. A+ strain hot-water (AH)	>100
P. A+ strain 70% ethanol (AE)	>100
P. natalensis cold-water (NC)	>100
P. natalensis hot-water (NH)	>100
P. natalensis 70% ethanol (NE)	>100
Doxorubicin	0.2169 ± 0.037

2.2. Effects of the Extracts on AngII-Induced Hypertrophy in H9C2 Cardiomyocytes

Morphological analysis of the actin filaments of the cardiomyocytes using rhodamine phalloidin reagent showed that AngII increased the cell size of the stimulated cardiomyocytes F-actin over 48 hours, Figure 1. Treatment with 50 µg/mL of the three extracts of *Pan cyanescens* (PC, PH and PE) and *P. cubensis* (GC, GH and GE) and the positive control losartan (100 µM) reduced the F-actin sizes of the cells, Figure 1.

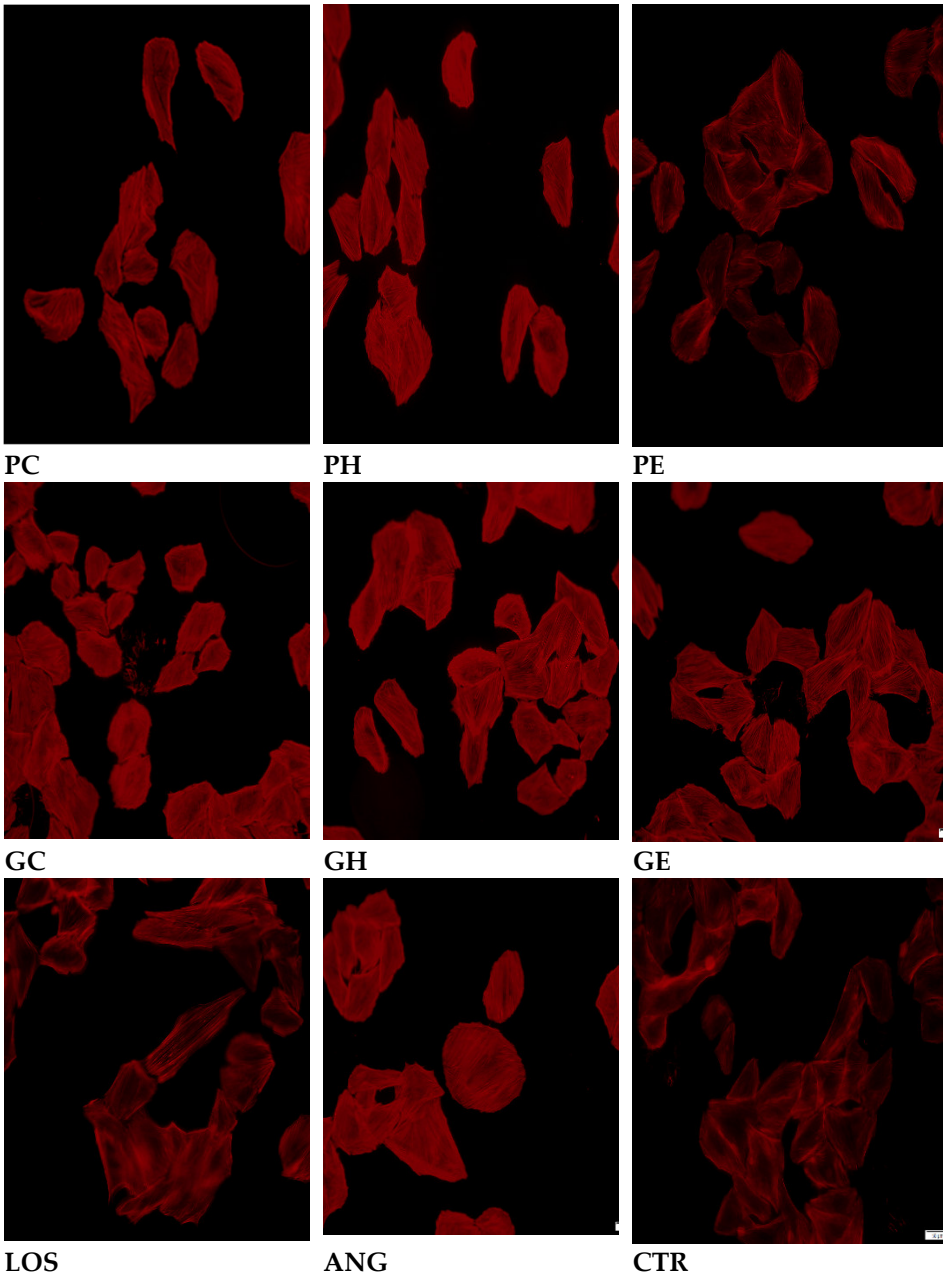

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Figure 1. Morphological effects of *Pan cyanescens* (PC, PH and PE) and *P. cubensis* (GC, GH and GE) mushroom extracts (50 µg/mL) and positive control losartan (LOS) (100 µM), non-induced negative control (CTR) on the actin filaments of AngII-induced (ANG) H9C2 cardiomyocytes (50 µm) over 48 hours using a fluorescence filter at Ex/Em= 546/575 nm.

Cell surface area measurements performed using CellSens Dimension 1,12 software on the cells showed in Figure 2 that AngII increased the cell width measurements of the induced cells 1.4-fold significantly ($p< 0.001$) compared to the non-induced negative control cells. The positive control losartan reversed these AngII effects significantly ($p< 0.001$). The cold-water (PC), hot-water (PH) and ethanol (PE) extracts of *Pan cyanescens* and *P. cubensis*'s cold-water (GC), hot-water (GH) and ethanol (GE) mushroom extracts also all reversed the AngII effects significantly, Figure 2.

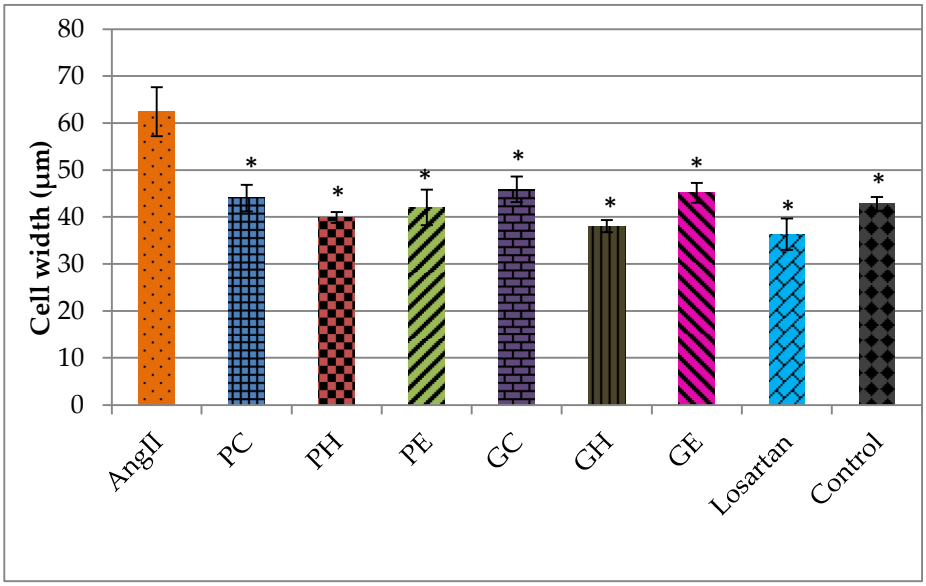
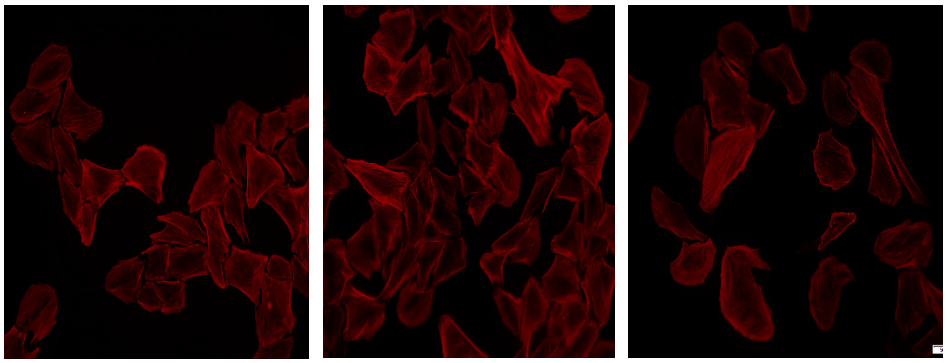


Figure 2. The effects of *Pan cyanescens* (cold-water PC, hot-water PH and ethanol PE) and *P. cubensis* (cold-water GC, hot-water GH and ethanol GE) mushroom extracts (50 µg/mL) and positive control Losartan (100 µM) on the cell width measurements on AngII-induced hypertrophy on H9C2 cardiomyocytes over 48 hours. Control: non-induced negative control. (*: significant).

Florescence morphological studies showed a decreased F-actin sizes in the AngII-induced cells treated with the cold-water, hot-water and ethanol extracts of *P. A+ strain* (AC, AH and AE) and *P. natalensis* (NC, NH and NE) mushrooms over 48 hours, in comparison to the AngII-stimulated cells, Figure 3.



AC **AH** **AE**

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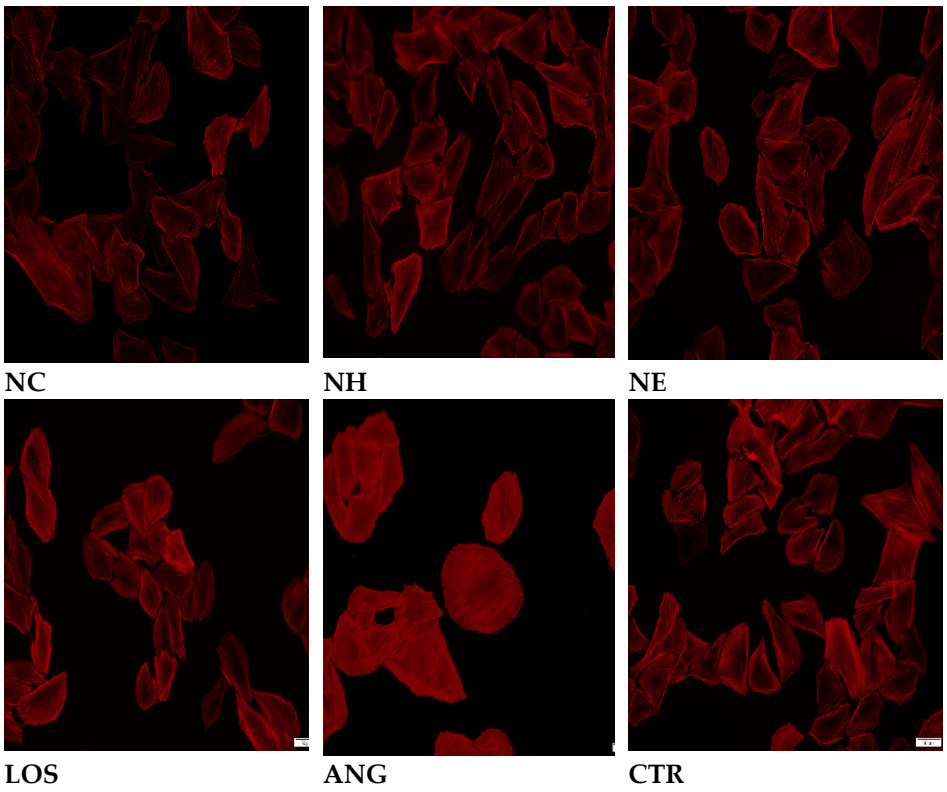

Figure 3. Morphological effects of *P. A+ strain* (AC, AH and AE) and *P. natalensis* (NC, NH and NE) mushroom extracts (25 µg/mL) and positive control losartan (LOS) (100 µM) on the actin filaments of AngII-induced H9C2 cardiomyocytes (50 µm) over 48 hours using a fluorescence filter at Ex/Em= 546/575 nm. CTR: non-induced negative control cells.

The treatment with the cold-water, hot-water and ethanol extracts of *P. A+ strain* (AC, AN and AE) and *P. natalensis* (NC, NH and AE) mushrooms reduced the AngII-induced cell size measurements significantly compared to the AngII-stimulated cells, Figure 4.

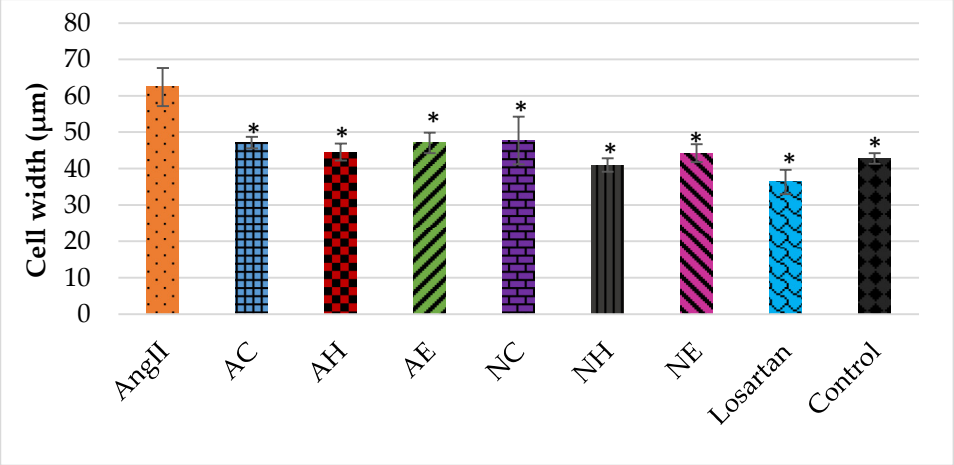

Figure 4. The effects of *P. A+ strain* (cold-water AC, hot-water AH and ethanol AE) and *P. natalensis* (cold-water NC, hot-water NH and ethanol NE) mushroom extracts (25 µg/mL) and positive control Losartan (100 µM) on the cell width measurements on AngII-induced hypertrophy on H9C2 cardiomyocytes over 48 hours. Control: non-induced negative control. (*: significant).

2.3. Effects of the Extracts on ANP Levels in AngII-Induced Cardiomyocytes

The AngII non-significantly increased the ANP levels of stimulated cells compared to the control cells. All the extracts of the four mushrooms lowered AngII-induced ANP concentration, however

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non-significantly, results not showed. Only the positive control, losartan reversed the AngII-induced ANP concentration significantly (p= 0.0093).

2.4. Effects of the Extracts on Mitochondrial Activity of AngII-Induced Cells

Angiotensin II stimulation reduced the mitochondrial activity indicated by lowering viability of cells significantly (p< 0.001) below 80% in comparison to the control cells, Figure 5. Positive controls, losartan and L-NAME increased the viability of AngII-induced cells in a concentration-dose manner and the effects were more pronounced with L-NAME treatment that restored viability of cells in line with control cells and above 100% cell viability with the 100 µM treatment.

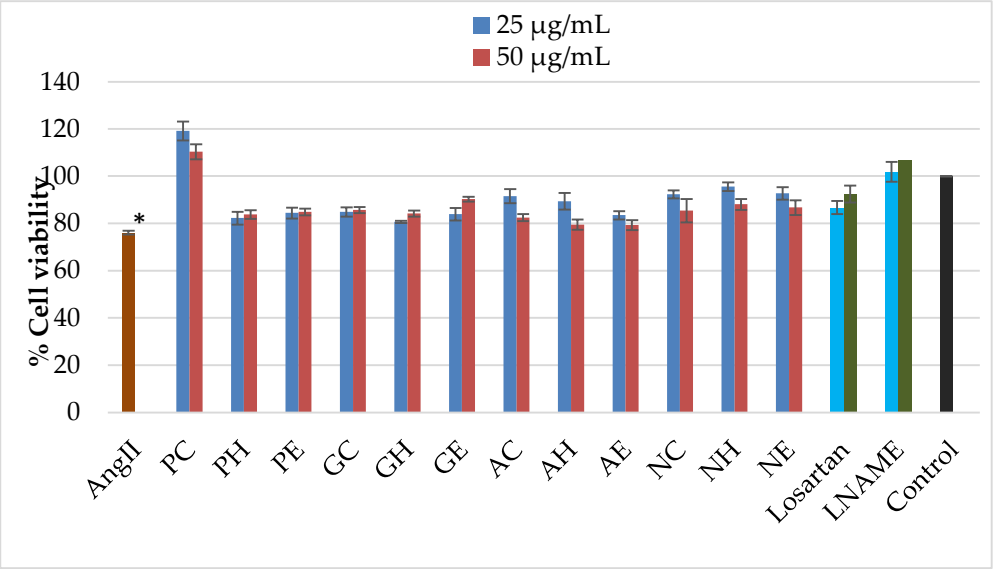

Figure 5. Effects of the extracts (25 and 50 µg/mL) of Pan cyanescens (cold-water PC, hot-water PH and ethanol PE), P. cubensis (cold-water GC, hot-water GH and ethanol GE), P. A+ strain (cold-water AC, hot-water AH and ethanol AE) and P. natalensis (cold-water NC, hot-water NH and ethanol NE) mushroom and positive controls; losartan and LNAME (50, 100 µM) on the mitochondrial activity of AngII-induced hypertrophy over 48 hours. Control: non-induced negative control. (*: significant).

The cold-water extract of *Pan cyanescens* increased viability of AngII-induced cells above LNAME the positive control and the control cells with the concentration investigated in the study(50 µg/mL and 25 µg/mL concentration), Figure 5. The hot-water and ethanol extracts of *Pan cyanescens* increased the viability same as the P. cubensis above 80% also in a dose dependant manner, Figure 5. *P. A+ strain* treatment increased the % viability of the cells above 80% with lowest concentration (25 µg/mL) investigated in the study while the higher 50 µg/mL concentration slightly increased the viability around and below 80% with the three extracts. The *P. natalensis* increased viability of cells above 80% in a concentration-dependent manner and to the same level as losartan with the 25 µg/mL concentration.

2.5. Effects of the Extracts on Intracellular ROS Levels in AngII-Induced Cardiomyocytes

Angiotensin II stimulation increased ROS levels significantly (p< 0.001) when compared with the non-induced but serum starved control cells, Figure 6. The cold-water, hot-water and ethanol extracts of all the extracts reduced the AngII stimulated ROS levels significantly similar to losartan (p< 0.001) when compared to AngII-induced cells.

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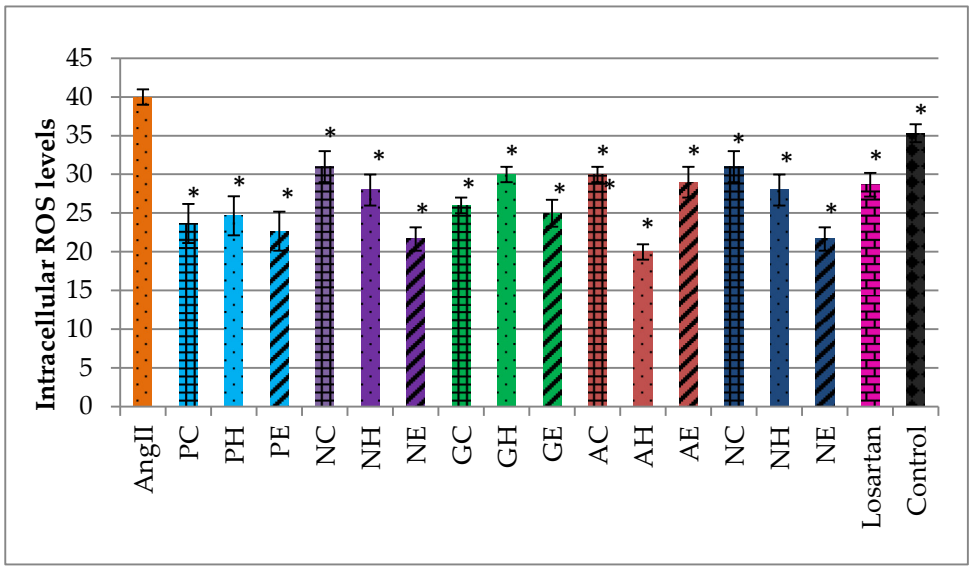

Figure 6. Effects of 1-hour treatment with 50 µg/mL extracts of *Pan cyanescens* (cold-water PC, hot-water PH and ethanol PE), *P. cubensis* (cold-water GC, hot-water GH and ethanol GE), and 25 µg/mL of *P. A+ strain* (cold-water AC, hot-water AH and ethanol AE) and *P. natalensis* (cold-water NC, hot-water NH and ethanol NE) and the positive control Losartan (100 µM) treatments on fluorometric intracellular ROS (superoxide and hydroxyl radicals) production measured using a green fluorescence intensity at λex= 485/20,520/25 nm on AngII-induced cardiomyocytes. Control: non-induced negative control. (*: significant).

2.6. Phytochemistry Analysis of the Water and Ethanol Extracts of Pan cyanescens, P. cubensis and A+ Strain Mushrooms

Figure 7 show the GCMS-MS chromatograms of the cold-water, hot-water and ethanol extracts of Pan cyanescens mushrooms respectively. The compounds with known anti0xidant and anti-inflammatory biological activities from the chromatograms of the three extracts are tabulated in Table 2 with their different peak numbers, compound name, molecular weight, formulas, similarity, area%, diemention time and height per extracts. Four compounds (n-hexadecanoic acid; 3-octanone, nonadane and tetradecane) with known natural antioxidant and anti-inflammatory activities similar to the ones extracted from *P. natalensis* identified in [21] were also present in the *Pan cyanescens* mushroom extracts. The compounds 9-Octadecenamide, (Z)- and n-hexadecanoic acid were found in all the extracts. Tetradecane was found in the hot-water extract and the ethanol extract. Nonadecane and decane were detected in the ethanol extract together with 9,12-Octadecadienoic acid (Z,Z)- and heineicosane. Dodecane, 1,1-dimethoxy- compound was detected in different peaks only with cold water together with hexanoic acid, methyl ester while dotriacontane compound and olean-12-ene-3,28-diol, (3á)- was detected only in the hot water extract of *Pan cyanescens,* Table 2.

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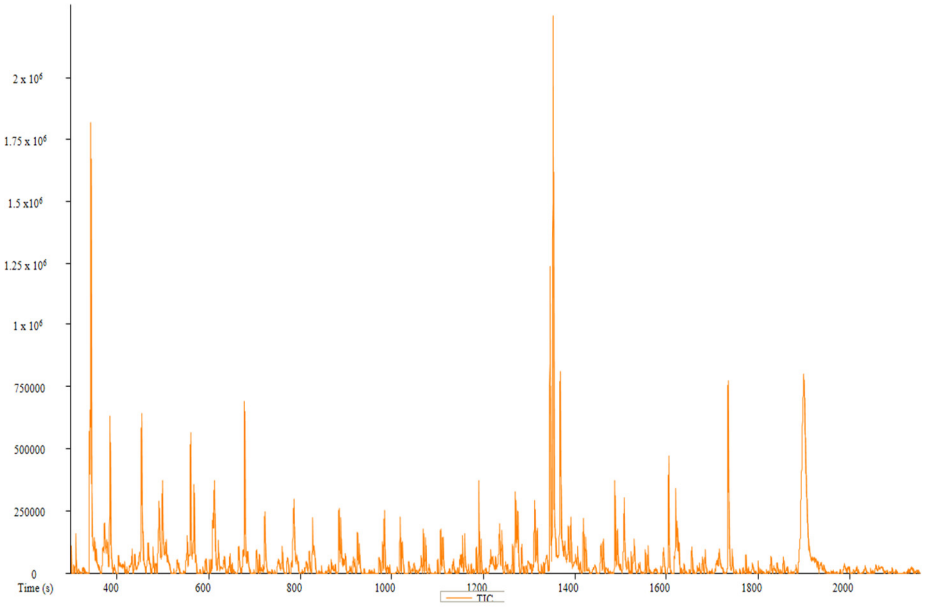
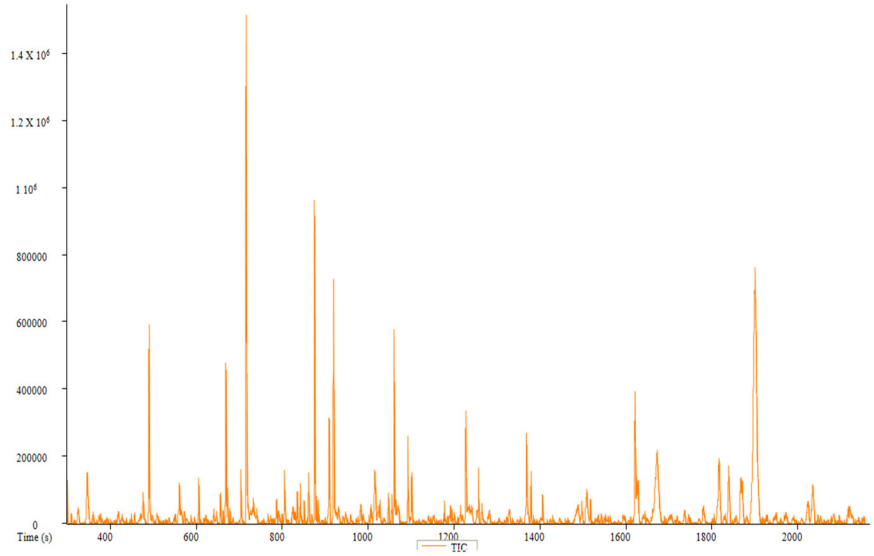


PC



PH

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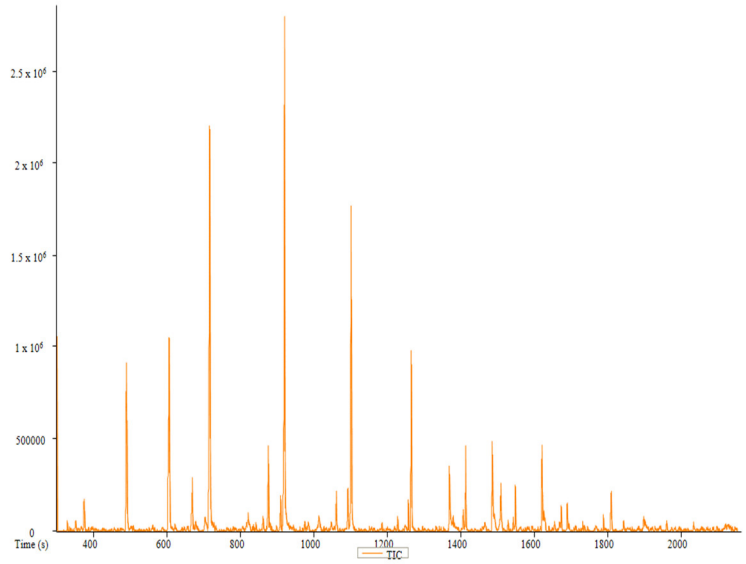

PE

Figure 7. The GCMS-MS chromatogram of cold-water (PC), hot-water (PH) and 70% ethanol (PE) extracts of Pan cyanescens mushroom.

Table 2. The compounds with anti-inflammatory and antioxidant effect identified in *Pan cyanescens* cold-water (PC), hot-water (PH) and 70% ethanol (PE) mushroom extracts.

PC	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	4	Hexanoic acid, methyl ester	130	C7H14O2	106-70-7	927	**2,0459**	385,6	614562
	6	3-Octanone	128	C8H16O	106-68-3	794	**2,9534**	453,6	647828
	7	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	705	**2,8617**	455	639963
	22	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	778	0,66502	785,8	265590
	26	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	816	0,79635	886	265712
	30	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	820	0,36008	980,6	132773
	35	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	843	0,4545	1070,3	185705
	38	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	815	0,46437	1155,4	155284
	42	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	794	0,93345	1236,3	196900
	43	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	800	0,56178	1241,5	176011
	47	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	781	1,0495	1313,4	297088
	51	n-Hexadecanoic acid	256	C16H32O2	57-10-3	899	**2,891**	1369	754734
	52	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	840	0,65301	1386,9	155364
	53	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	774	0,66727	1392,4	200963
	58	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	798	0,22375	1524,4	90547
	62	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	908	1,1739	1621,3	345374
	63	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	816	0,81034	1624,9	215839

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	64	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	733	0,51097	1628,1	131186
	66	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	770	0,35671	1716,6	102006
	68	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	791	0,20132	1774,1	81447
PH	**Peak #**	**Name**	**Weight**	**Formula**	**CAS**	**Similarity**	**Area %**	**1st Dimension Time (s)**	**Height**
	13	Tetradecane	198	C14H30	629-59-4	884	**4,0398**	920,1	731151
	19	n-Hexadecanoic acid	256	C16H32O2	57-10-3	868	1,9932	1369,2	270343
	22	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	921	**2,6151**	1621,3	395816
	23	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	829	0,98533	1625	141739
	24	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	877	1,1489	1628,4	130670
	26	Dotriacontane	450	C32H66	544-85-4	885	**5,8278**	1673,2	221028
	32	Olean-12-ene-3,28-diol, (3á)-	442	C30H50O2	545-48-2	613	**19,938**	1900,8	766581
PE	**Peak #**	**Name**	**Weight**	**Formula**	**CAS**	**Similarity**	**Area %**	**1st Dimension Time (s)**	**Height**
	5	Decane	142	C10H22	124-18-5	846	**8,8254**	605,8	1051849
	10	Tetradecane	198	C14H30	629-59-4	880	**18,263**	919,9	2801754
	14	Nonadecane	268	C19H40	629-92-5	893	**10,729**	1101,5	1772907
	15	Nonadecane	268	C19H40	629-92-5	904	**5,3724**	1265	980759
	17	n-Hexadecanoic acid	256	C16H32O2	57-10-3	880	**2,671**	1369,1	353542
	19	9,12-Octadecadienoic acid (Z,Z)-	280	C18H32O2	60-33-3	910	**3,4485**	1485,3	489829
	22	Heneicosane	296	C21H44	629-94-7	899	1,3394	1548,6	251573
	23	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	924	**2,509**	1621,2	466852

Figure 8 show the GCMS-MS chromatograms of the cold-water, hot-water and ethanol extracts of *P. cubensis* mushrooms respectively. The compounds with known biological activities from the chromatograms of the three extracts are tabulated in Table 3 with their different peak numbers, compound name, molecular weight, formulas, similarity, area%, diemention time and height per extracts. Four compounds (n-hexadecanoic acid; 3-octanone, nonadane and tetradecane) with natural antioxidant and anti-inflammatory activities similar to the ones extracted from *P. ntalensis* identified in [21] were also present in these mushroom extracts, Table 3. The compound n-hexadecanoic acid was present in all the extracts while tetradecane compound was detected only in the ethanol extract together with hexadecane, 9,12-Octadecadienoic acid (Z,Z)-, nonadecane, heneicosane, oleic acid and decane. Compound 9-Octadecenamide, (Z)- was detected in different peaks in both ethanol and hot-water extracts. In the cold and hot-water extracts, dodecane, 1,1-dimethoxy- was detected in many different peaks and hexadecanoic acid, methyl ester; 3-octanone and dodecanoic acid, methyl ester compounds were also detected in the extract.

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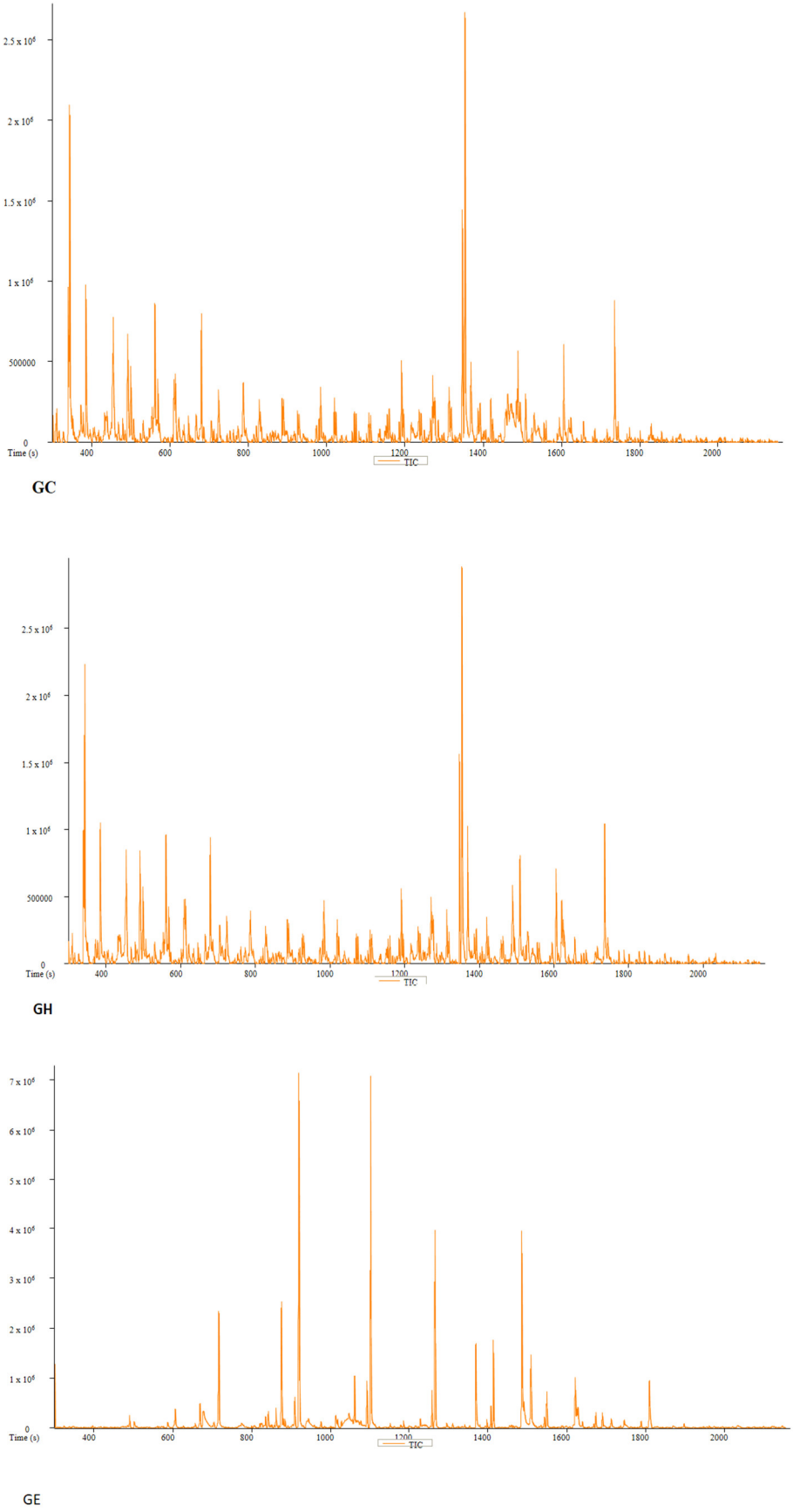

Figure 8. The GCMS-MS chromatogram of cold-water (GC), hot-water (GH) and 70% ethanol (GE) extracts of P. cubensis mushroom.

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Table 3. The compounds identified in *P. cubensis* cold-water (GC), hot-water (GH) and 70% ethanol (GE) mushroom extracts.

GC	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	3	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	739	**6,3304**	343,2	2096696
	6	Hexanoic acid, methyl ester	130	C7H14O2	106-70-7	928	**2,8062**	385,1	955717
	9	3-Octanone	128	C8H16O	106-68-3	830	**2,7283**	452,8	762082
	27	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	779	0,71327	785,7	314589
	31	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	813	0,69149	885,8	276576
	35	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	855	0,35927	980,5	151860
	38	Dodecanoic acid, methyl ester	214	C13H26O2	111-82-0	900	0,70445	1019,3	279675
	41	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	807	0,44597	1070,2	197083
	48	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	809	0,78095	1236,2	194599
	53	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	736	1,2754	1313,2	346174
	55	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	910	**3,7206**	1346,9	1438966
	57	n-Hexadecanoic acid	256	C16H32O2	57-10-3	876	1,7469	1368,7	479084
	58	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	837	0,81401	1386,7	214202
	59	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	761	0,89417	1392,1	245098
	65	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	851	0,21253	1524,3	93108
	71	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	729	0,20497	1716,4	85138
GH	**Peak #**	**Name**	**Weight**	**Formula**	**CAS**	**Similarity**	**Area %**	**1st Dimension Time (s)**	**Height**
	3	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	718	**5,7808**	343,8	2236529
	11	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	739	**3,5171**	454,8	833100
	20	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	745	0,93596	569	403943
	28	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	759	0,65184	785,8	327037
	32	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	827	0,73365	885,8	331466
	37	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	833	0,44286	980,5	180459
	40	Dodecanoic acid, methyl ester	214	C13H26O2	111-82-0	862	0,76451	1019,3	332902
	46	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	813	0,47338	1155,3	187669
	48	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	829	1,4125	1191,3	568445
	50	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	802	0,88819	1236,2	252623
	55	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	784	1,2221	1313,2	405746
	56	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	789	0,80275	1318,5	238850

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59	n-Hexadecanoic acid	256	C16H32O2	57-10-3	891	**3,1125**	1368,8	1020178
60	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	827	0,72977	1386,7	208562
61	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	772	0,67475	1392,2	260361
69	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	845	0,23403	1524,2	119865
74	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	901	1,5525	1620,9	476077
75	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	828	0,94615	1624,4	327188
76	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	768	0,71306	1627,7	212987
78	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	839	0,41231	1716,3	134307
80	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	772	0,22056	1773,7	103514
81	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	735	0,27314	1829,2	96189

GE	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	1	Decane	142	C10H22	124-18-5	897	0,44944	490,3	252016
	3	Decane	142	C10H22	124-18-5	842	0,98073	605,7	379188
	8	Tetradecane	198	C14H30	629-59-4	864	**4,5504**	716,3	2342975
	14	Tetradecane	198	C14H30	629-59-4	873	**12,505**	919,9	7151813
	20	Hexadecane	226	C16H34	544-76-3	914	**11,926**	1101,7	7087174
	24	Nonadecane	268	C19H40	629-92-5	919	**6,5048**	1265	3973633
	26	n-Hexadecanoic acid	256	C16H32O2	57-10-3	916	**3,2652**	1368,9	1698554
	29	Heneicosane	296	C21H44	629-94-7	901	**2,7681**	1413,3	1765288
	30	9,12-Octadecadienoic acid (Z,Z)-	280	C18H32O2	60-33-3	920	**7,7412**	1485,4	3942406
	31	Oleic Acid	282	C18H34O2	112-80-1	901	1,7768	1490,7	498823
	32	Dodecanamide	199	C12H25NO	1120-16-7	822	0,1794	1506,4	175567
	36	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	920	**2,013**	1620,9	1001169
	37	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	875	1,1399	1627,8	415638
	42	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	827	0,33757	1745	161336

Figure 9 show the GCMS-MS chromatograms of the cold-water, hot-water and ethanol extracts of P. A+ *strain* mushrooms respectively while the compounds with know biological activities from the chromatograms of the three extracts are tabulated in Table 4 with their molecular weight, formulas and area% per extracts. The four compounds (n-hexadecanoic acid; 3-octanone, nonadane and tetradecane) with natural antioxidant and anti-inflammatory activities similar to the ones extracted identified in [21] were also present in the *P. A+ strain* mushroom extracts. The compound n-hexadecanoic acid was detected in all the extracts of *P. A+ strain*. Decane, tetradecane, 9,12-octadecadienoic acid (Z,Z)-, and eicosane compounds were detected in the ethanol extract. The compounds hexadecanoic acid, methyl ester, 3-Octanone and 9-octadecenamide, (Z)-, were detected in both cold and hot water extracts with dodecane, 1,1-dimethoxy detected in many different peaks in both water extracts of *P. A+ strain* mushroom while glycine was detected only in the hot-water extract.

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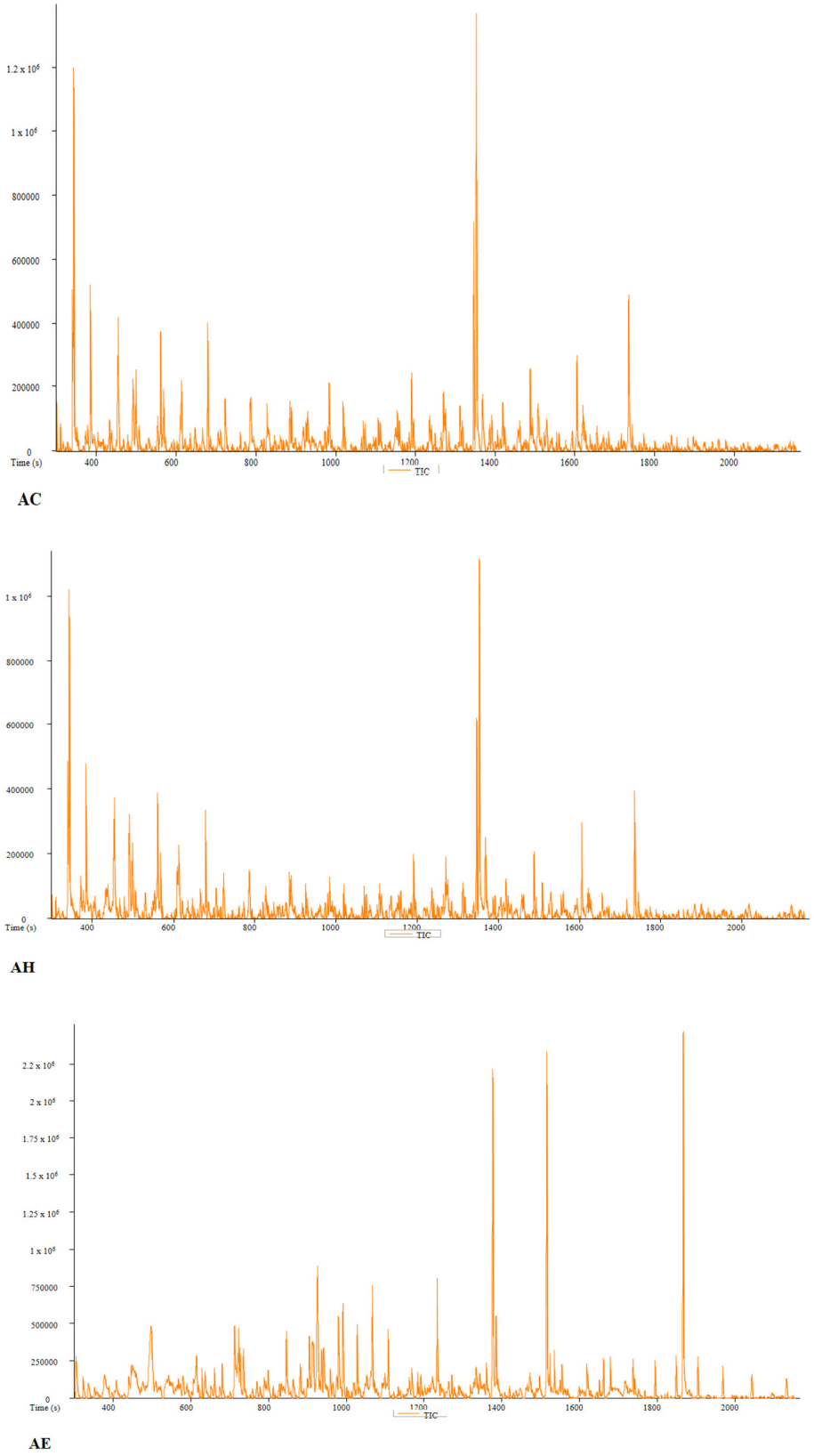

Figure 9. The GCMS-MS chromatogram of cold-water (AC), hot-water (AH) and 70% ethanol (AE) extracts of P. A+ strain mushroom.

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Table 4. The compounds identified in P. A+ strain cold-water (AC), hot-water (AH) and 70% ethanol (AE) mushroom extracts.

AC	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	3	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	732	**7,2746**	343,1	1201850
	5	Hexanoic acid, methyl ester	130	C7H14O2	106-70-7	928	**3,7608**	385	524718
	7	3-Octanone	128	C8H16O	106-68-3	852	**3,7989**	452,8	422676
	8	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	725	**3,7989**	454,5	422676
	24	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	742	0,77714	785,7	152661
	27	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	805	1,1092	885,8	160107
	30	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	841	0,47523	980,5	80179
	35	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	850	0,5429	1070,1	98825
	38	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	810	1,0146	1155,1	128129
	39	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	748	0,71579	1160,2	83648
	41	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	829	1,066	1236,1	112539
	45	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	754	1,2357	1313,2	144052
	46	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	732	0,83455	1318,6	97752
	47	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	910	**4,4828**	1347	720709
	49	n-Hexadecanoic acid	256	C16H32O2	57-10-3	884	**2,0361**	1369,1	181013
	50	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	802	0,68996	1386,7	85828
	60	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	895	1,3478	1621	146411
	61	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	764	0,62809	1624,5	93200
	63	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	859	0,36481	1716,4	56531
AH	**Peak #**	**Name**	**Weight**	**Formula**	**CAS**	**Similarity**	**Area %**	**1st Dimension Time (s)**	**Height**
	3	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	729	**7,5091**	343,1	1023514
	6	Hexanoic acid, methyl ester	130	C7H14O2	106-70-7	942	**3,4369**	384,8	481718
	8	3-Octanone	128	C8H16O	106-68-3	822	0,94495	452,6	
	9	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	711	**4,0912**	454,3	366395
	26	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	783	0,82861	785,6	133376
	30	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	817	1,059	885,7	146601
	33	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	854	0,51922	980,3	80840
	38	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	827	0,58444	1070,1	106177
	41	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	817	0,60789	1155,2	76344
	43	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	851	1,3131	1191,4	202951
	44	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	828	1,0656	1236,1	99142

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45	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	779	0,30158	1241,2	66062
48	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	773	1,0275	1313,2	116536
50	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	910	**3,9708**	1347,1	619698
52	n-Hexadecanoic acid	256	C16H32O2	57-10-3	894	**2,3541**	1368,8	236893
53	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	831	0,54835	1386,7	69077
54	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	740	0,54021	1392,2	75964
59	Glycine	75	C2H5NO2	56-40-6	754	1,0692	1530,1	87122
63	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	864	0,62408	1621,2	97425
65	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	808	0,58739	1716,4	61884

AE	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	10	Decane	142	C10H22	124-18-5	933	**20,924**	497,5	783673
	31	Tetradecane	198	C14H30	629-59-4	888	**3,381**	925,5	289073
	39	n-Hexadecanoic acid	256	C16H32O2	57-10-3	911	**3,0487**	1375,1	418335
	37	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	851	0,54568	1353	74443
	42	9,12-Octadecadienoic acid (Z,Z)-	280	C18H32O2	60-33-3	914	**2,1293**	1491,4	204219
	35	Eicosane	282	C20H42	112-95-8	896	1,4654	1107,3	168172

In Table 5 below, there are other compounds that were extracted and not included in Nkadimeng et al 2020 [21] and are tabulated here. Compound heneicosane, 9,12-octadecadienoic acid (Z,Z)-, 5-eicosene, (E)- and decane were detected only in the ethanol extract. Dodecane, 1,1-dimethoxy- was detected in many different peaks in both hot and cold-water extracts while hexadecanoic acid, methyl ester and dodecanoic acid, methyl ester was only detected in the cold-water extracts of *P. natalensis* mushroom.

Table 5. Other compounds identified in *P. natalensis* cold-water, hot-water and 70% ethanol mushroom extracts. (Chromatograms published in [18]).

NH	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	7	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	715	**3,2977**	454,6	567775
	23	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	817	1,1041	885,9	210887
	26	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	830	0,42934	980,6	105250
	28	Dodecanoic acid, methyl ester	214	C13H26O2	111-82-0	907	1,1472	1019,6	224678
	30	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	843	0,63486	1070,3	134265
	33	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	810	0,86841	1155,4	139945
	36	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	806	0,92241	1236,2	152342
	37	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	821	0,4793	1241,4	120478

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40	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	750	1,677	1313,3	251622
45	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	809	1,1774	1386,7	152379
46	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	777	0,97402	1392,3	175193
50	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	795	0,26298	1524,4	67498
55	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	860	1,1976	1621,4	205738
57	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	771	0,27229	1716,4	72493
59	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	731	0,47328	1773,9	89699

NC	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	13	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	733	**3,6742**	454,9	751534
	31	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	779	0,68667	785,7	315435
	33	Decanoic acid, methyl ester	186	C11H22O2	110-42-9	890	0,63556	827,8	263610
	35	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	831	0,74448	885,8	274267
	40	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	837	0,43806	980,5	161533
	43	Dodecanoic acid, methyl ester	214	C13H26O2	111-82-0	913	0,67789	1019,4	277970
	46	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	797	0,44929	1070,1	184949
	49	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	789	0,58295	1155,3	183945
	53	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	783	0,87504	1236,2	203315
	58	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	751	0,94274	1313,2	320486
	59	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	809	0,79948	1318,7	210365
	63	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	820	0,75158	1386,7	170332
	64	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	759	0,71847	1392,2	224832
	65	Hexadecanoic acid, methyl ester	270	C17H34O2	112-39-0	844	0,87915	1419,5	273632
	70	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	836	0,24407	1524,3	103354
	73	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	758	0,50675	1594,8	141017

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75	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	889	0,73559	1621,2	243511
76	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	713	0,68859	1624,7	202379
78	Dodecane, 1,1-dimethoxy-	230	C14H30O2	14620-52-1	776	0,52186	1656,7	149748

NE	Peak #	Name	Weight	Formula	CAS	Similarity	Area %	1st Dimension Time (s)	Height
	2	Decane	142	C10H22	124-18-5	874	1,1285	490	365956
	17	5-Eicosene, (E)-	280	C20H40	74685-30-6	904	1,1568	1257,2	551903
	23	5-Eicosene, (E)-	280	C20H40	74685-30-6	904	0,76517	1406,5	329793
	24	Heneicosane	296	C21H44	629-94-7	901	3,1921	1413,2	1359751
	25	9,12-Octadecadienoic acid (Z,Z)-	280	C18H32O2	60-33-3	884	1,6761	1485,1	574310
	27	Oleic Acid	282	C18H34O2	112-80-1	828	1,6454	1508,6	534460
	28	Dotriacontane	450	C32H66	544-85-4	921	1,3086	1548,6	624893
	29	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	927	**2,1573**	1621	787743
	30	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	857	0,99448	1624,7	316129
	31	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	847	1,0811	1627,8	304295
	35	9-Octadecenamide, (Z)-	281	C18H35NO	301-02-0	827	0,26441	1745	100584

3. Discussion

Previous studies have demonstrated that AngII plays a key role in progression of heart failure by affecting cell growth, differentiation and apoptosis, induction of pro-inflammatory cytokines and many other factors in cardiomyocytes [14]. In the study, morphological F-actin size results and cell width measurements showed that the cells that were induced with AngII increased the cell surface area of the cells significantly compared to non-induced serum starved control cells in agreement with previous studies [19]. Angiotensin II stimulation decreased mitochondrial activity by lowering cell viability < 80% signifying cell death and also increased levels of ROS production significantly while ANP level was non-significantly increased in cardiomyocytes. The results showed that the positive control losartan, which is an AngII inhibitor via blockage of ATR1 receptors, significantly reduced the AngII-induced cell surface area measurements, ROS and ANP levels of the stimulated cells. Losartan also improved cell viability of AngII-induced cells in a dose-dependent manner similar to L-NAME. This study showed that L-NAME, which is a non-selective NOS inhibitor, prevented the AngII-induced cell death in greater percentage indicating involvement of NOS uncoupling in the AngII-induced injury and cell death in the study.

Our study demonstrated that the cold-water, hot-water and ethanol extracts of *Pan cyanescens*, *P. cubensis*, *P. A+ strain* and *P. natalensis* mushroom extracts alleviated the cell enlargement induced

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by AngII stimulation same as the positive control, losartan. Cell enlargement is one of the key indices of hypertrophy and by reducing it, the extracts demonstrated not just safety but potential protective effects as well in AngII-induced hypertrophy conditions. The concentrations of ANP, which is a maker in heart failure, was also found to be lower however non-significant in the three extract-treatments. Since the increase levels of ANP are known to be associated with the severity of heart failure condition, their decrease agreed with the significant decrease in cell size enlargement supporting alleviation of hypertrophy observed with the mushroom extracts' treatments.

Other well-investigated pro-hypertrophic mechanisms of AngII are also known to be mediated via activation of NAD(P)H oxidase through ATR1 receptors and inducing mitochondrial and induced-cell death leading to increased ROS generation and more oxidative stress [14,15]. In our study, we measured intracellular ROS especially superoxide and hydroxyl radicals both of which are known to increase significantly with AngII stimulation [13]. Our study showed that the cold-water, hot-water and ethanol extracts of all the four magic mushrooms alleviated these AngII-induced intracellular ROS generation of treated cells significantly similar to Losartan. By reducing accumulation of AngII-induced ROS, the extracts of *Pan cyanescens*, *P. cubensis*, *P A+ strain* and *P. natalensis* mushrooms demonstrated safety and protective potentials of the extracts in AngII-induced oxidative stress conditions in vitro in cardiomyocytes in the concentration investigated. These results agreed with our previous finding in endothelin-induced ROS activity following treatment with *Pan cyanescens, P. cubensis* water extracts [20]. Furthermore, we have also demonstrated invitro anti-inflammatory potential of the four extracts on LPS-induced human macrophage cells [21].

In addition, the four mushroom extracts also protected against AngII-induced mitochondrial and cell death signified by increasing in % viability of cells above 80% in safe margins with the concentrations investigated 50 µg/mL for Pan cyanescens and P. *cubensis*, and 25 µg/mL for *P. A+ strain* and *P. natal*ensis mushrooms in the study. However, the study also showed that *P. A+ strain* mushroom extracts may be toxic if higher than 50 µg/mL concentrations are used in an AngII pathological condition.

The cytotoxicity assay results on H9C2 cardiomyocytes also showed that the three extracts of the four magic mushrooms were not toxic when compared to the positive control doxorubicin. The extracts were safe in the order ethanol> hot-water> cold-water for the three magic mushrooms and the order hot-water> ethanol> cold-water for *P. cubensis*. Moreover, in accordance to the American National Cancer Institution guidelines, it is indicated that extracts that exhibit an LC50 ≤ 20 µg/mL over 48 hours treatment are considered toxic. As a result, the three extracts of the four mushrooms will be considered to be safe with LC50 presented and also with the 50 µg/mL and 25 µg/mL concentration which were investigated in the study. However, further investigations in vivo to confirm this safety is recommended.

The phytochemistry analysis of the water and ethanol extracts showed presence of known natural compounds with antioxidant and anti-inflammatory activities in support of these ant-oxidative stress effects observed in the study. The two compounds, 9-Octadecenamide, (Z)- which is a potent antioxidant with antimicrobial activities, and n-hexadecanoic acid which is the most common saturated fatty acid known to have anti-inflammatory and antioxidant activities were detected in all the water and ethanol extracts of the four magic mushrooms [22–24].

Decane, an alkaline hydrocarbon compound, was found to possess activities such as phosphatase and membrane permeability inhibitor which means it prevents damage and preserve integrity of the cellular membrane [25]. The compound was also reported as platelet aggregation inhibitor and platelet activating factor beta antagonist which are known to inhibit thrombus production by decreasing platelet agglutination with potential therapeutic agents in different diseases including cardiovascular [25]. This compound was detected only in all the ethanol extracts of the four psilocybin mushrooms.

Tetradecane is an alkaline hydrocarbon compound which was found to have anti-inflammatory effects and also a potential membrane integrity agonist, cardiovascular analeptic (which are central nervous system stimulants agents that increases alertness, heart rate, blood pressure, breathing and blood glucose level, mood and euphoria among others) and nicotinic alpha6beta3beta4alpha5

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receptor antagonist [25,26]. This compound was detected in all the ethanol extracts of the four psilocybin mushrooms extracts.

The three compounds, nonadecane, an alkaline hydrocarbon lipid molecule and vey hydrophobic with antioxidant, antibacterial and antimalarial activities [26–28]; 9,12-Octadecadienoic acid (Z,Z)- compound which has been reported to have antioxidant activity, and heneicosane, an aliphatic hydrocarbon compound that is reported to complements C5a chemotactic receptor antagonist which play important roles in inflammation and cell killing process [29], and also to possess anti-eczema atopic activities, phobic disorders treatment and betaadrenergic receptor kinase inhibitor which are known to ameliorate cardiac dysfunction and improve survival especially in heart failure [25] were all detected only in the ethanol extracts of the three psilocybin mushrooms, *P. natalensis, Pan cyanescens* and *P. cubensis* and it was not detected in any of the P. A+ strain mushroom extracts.

Hexadecanoic acid, methyl ester which is known to have antioxidant, anti-inflammatory (by inhibiting cyclooxygenase-2 enzymes) activities and also a blood cholesterol decreasing effect [30] was detected in the water extracts of *P. A+ strain* and *P. cubensis*, and also in the cold-water extracts of *Pan cyanescens* and *P. natalensis* mushrooms. Compound 3-Octanone reported to have antioxidant and anti-inflammation activities was detected in the water extracts of *P. cubensis, P. A+ strain* and *P. natalensis* mushrooms and in the cold water of Pan cyanescens mushroom [18,31]. Dotriacontane reported to have antioxidant activities were detected in the ethanol extracts of *P. cubensis* and *P. natalensis* mushrooms and also in the hot-water extracts of *Pan cyanescens* mushroom extracts [32].

Hexadecane has been found to have antibacterial, cognition disorder treatment, antianginal, nicotinic alpha6beta3beta4alpha5 and nicotinic alpha2beta2 receptor antagonist, kidney function stimulant and 5-hydroxytryptamine uptake stimulant. This compound was detected in ethanol extracts of *P. cubensis* [25,33]. Oleic acid is a mono-unsaturated omega9-fatty acid known to enhance antioxidant activity,inhibit adrenoleukodystrophy, boost memory, a key factor accounting for the hypotensive effects of olive oil [34] and generally known to improve and protect against cardiovascular disease [35], 2021) was detected in ethanol extracts of *P. cubensis*. Glycine, which is known to improves the body's ability to use nitric oxide and relief blood pressure [36] was detected only in the hot-water extract of *P. A+ strain* mushroom. Olean-12-ene-3,28-diol, (3á)-, which is known to have anti-inflammatory and protecting activities against induced experimental autoimmune or allergic encephalomyelitis [37] was detected in the hot water extracts of *Pan cyanescens* mushroom extract.

In summary, the study showed that AngII induced cell enlargement and ANP levels signifying hypertrophy in the stimulated cells. Angiotensin II stimulation also induced cell injury and death by decreasing cell viability and increasing ROS generation and NOS activity in the induced cells. Losartan, the positive control reversed these AngII-induced hypertrophy effects and also cell injury effects similar to L-NAME in agreement with previous studies. The cold-water, hot-water and ethanol extracts of *Pan cyanescens, P. cubensis, P. A+ strain* and *P. natalensis* mushrooms reversed the cell size enlargement significantly and non-significantly lowered ANP levels (indices of AngII-induced hypertrophy effects) and protected the cardiomyocytes significantly against the AngII-induced oxidative stress and cell death in a manner similar to losartan at the 50 µg/mL (used for *Pan cyanescens and P. cubensis*) and 25 µg/mL (used for the *P. A+ strain and P. natalensis*) in the study. These findings suggested potential presence of compounds with antioxidant abilities known to neutralize the free radicals and alleviate intracellular ROS accumulation in the four mushrooms. The phytochemical analysis of the extracts confirmed these effects by showing detection of known compounds with antioxidant and anti-inflammatory effects in the water and ethanol extracts of these four psilocybin mushrooms.

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4. Materials and Methods

4.1. Ethical Clearances

The protocol for this study was approved by the University of Pretoria research committee with the number REC045-18. The project was also approved by the Medical Control Council (MCC) of the South African Health Department with a permit license POS 223/2019/2020 since psilocybin mushrooms are schedule 7 substances in South Africa.

4.2. Growing Mushrooms and Making Extracts

The spores print syringe of Panaeolus (*Copelandia*) cyanescens (*Pan cyanescens*), Psilocybe nataleases (*P. natalensis*), Psilocybe cubensis (*P. cubensis*), and Psilocybe cubensis leucistic A+ strain (P. A+ strain) mushrooms were verified by the Sporespot Company and sterile substrate and they were grown and extracted with 70% ethanol, cold and hot-boiling water as described in [18].

4.3. Culturing of Cells

The rat H9C2 cardiomyoblast cells were obtained from American Type Culture Collection (ATCC ® CRL-1446™) and maintained using Dolbecco Modified Eargle media (DMEM) (Pan, Separations Scientific) supplemented with 10% fetal bovine serum (FBS) (Gibco, Sigma Aldrich) and 1% of 100 IUnits/mL penicillin and 100 µg/L streptomycin (Pan, Celtics diagnostic) in 75 cm2 tissue culture treated flasks (NEST, Whitehead Scientific). The cells were grown in an incubator (HERAcell 150, Thermo Electron Corporations, USA) at 370C in 5% CO2 balanced air.

4.3.1. Cytotoxicity Determination of the Mushroom

The cytotoxicity of the extracts was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay described by Mosmann (1983) with modifications by Nkadimeng et al. (2020a). When grown to confluence the H9C2 cardiomyocytes were washed with pre-warmed phosphate buffer (PBS) (Sigma-aldrich), passaged with trypsin-EDTA (Biochom biotch) and neutralised with DMEM. The cells were centrifuged for 7 minutes and pellet resuspended with 1 mL of fresh medium. Thereafter cells were counted and seeded 1 × 104 cells (all the wells in column 2 to 12) in a 96 well tissue culture treated plates (NEST, Whitehead Scientific). After 24 hours cells had adhered fully, and medium was removed and replaced with fresh media 100 µl per well. Then the cells in column 2 to 10 were treated with the mushroom extracts (0.0075, 0.01, 0.025, 0.05, 0.075 and 0.1 µg/mL concentrations) and doxorubicin chloride (Pfizer Laboratories), a well-known toxic drug was used as a positive control (2, 4, 10 and 20 µM). Plates were incubated for 48 hours at 370C in 5% CO2 incubator. The wells of the 1st column without cells were used as blank while the wells of the last two columns 11 and 12 which were not treated were used as negative controls.

After 48 hours, medium was removed, and the cells were washed with 200 µl of pre-warmed PBS. Then 100 µl of fresh medium was added and 30 µl of MTT (Inqaba biotec, stock solution of 5 mg/ml in PBS) was added and the plates were incubated for 4 hours in dark at 370C in 5% CO2 incubator. After 4 hours, the media with MTT was removed and the formazon salts were dissolved with 50 µl of dimethyl sulfoxide (DMSO) (Sigma-aldrich) in dark. The plates were shaken for 1 minute and plates read using a microplate reader (Biotek, Synergy HT) at a wavelength of 570 nm and a reference wavelength of 630 nm. Untreated cells (negative control) were included. The treatment was performed in triplicates and the experiments were repeated three times. Viability of cells in percentages was calculated using the formula: % Viability= ((Sample Absorbance/control Absorbance) x 100). The results were expressed as lethal concentration (LC) LC50 value which is the concentration of the sample necessary to kill viability of cells by 50%.

4.3.2. Cell Culture for Treatment

The cells were cultured according to the method of [19] with modification. Briefly as soon as cells reached 70% confluence they were passaged, counted and 1 × 106 cells seeded and grown on

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glass cover slips in 6 well plates (NEST, Whitehead scientific). After 24 hours medium was removed, the cells in the 6 well plates were washed with 1 mL serum free DMEM and deprived of serum for 18 hours by adding 2 mL of serum-free DMEM. After 18 hours sera-free media was removed, and the cells were treated AngII (10 μM) (Sigma-aldrich) and incubated for 45 min before treated with the three extracts (50 μg/mL) of *Pan cyanescens* and *P. cubensis* and the three extracts (25 μg/mL) of *P. A+ strain* and *P. natalensis*, and positive control 100 μM Nω-nitro-L-arginine methyl ester (L-NAME, Sigma-aldrich) over 48 hours in 1% FBS media. Losartan100 μM (Sigma-aldrich), another positive control which is the selective AT1R inhibitor was induced 45 minutes prior to stimulation with AngII and treated over 48 hours in media supplemented with 1% FBS and 1% penicillin-streptomycin. This medium was used to prepare and dilute all the treatment and drugs. Stock concentrations of chemicals were prepared in sterile pure water. AngII cells were induced with AngII but not treated, while the control cells were serum starved but neither induced with AngII nor treated.

Mitochondrial Activity

To test for mitochondrial activity, 1 × 104 cells were seeded in 96 well plates (NEST, Whitehead scientific), deprived of serum the same way as above by removing old medium after 24 hours and adding 100 μl of serum-free DMEM over 18 hours prior to inducing the cells with 10 μM AngII for 45 minutes. Then cells were treated with the three extracts and positive controls over 48 hours in the presence of 1% FBS DMEM as above. AngII cells were cells that were induced with AngII but not treated and control cells were only serum starved but not stimulated with AngII nor treated. Mitochondrial activity was measured using the Resazurin assay kit AR002 (R & D, Whitehead scientific) according to the manufacture manual. The viability of cells in percentages was calculated using the formula: % Viability= ((Sample Absorbance/control Absorbance) x 100). The experiments were performed in triplicate and repeated in three different times.

Actin Filament Labelling and Surface Area Measurements

After 48 hours of treatment, the cells on coverslips in the 6 well plates were prepared for rhodamine phalloidin reagent fluorescence staining using ab235138 Rhodamine Phalloidin Reagent (Biocom Africa) for labeling, identifying and quantifying actin filaments (F-actin) in the cardiomyocyte cells according to the manual. Briefly the cells on coverslip were washed with PBS. Then cells were fixed with 3.5 % formaldehyde fixation in PBS at room temperature for 20 minutes. Formaldehyde solution was aspirated carefully, and cells washed with PBS. Then 0.1%Triton X-100 in PBS was added to the cells to increase permeability for 5 minutes and then washed with PBS. Then 1X phalloidin conjugate working solution was added into each well of fixed cells and incubated for 90 minutes. After 90 minutes, excess phalloidin conjugate was removed and mounting media added to preserve fluorescence and sealed. The cells were then observed using a fluorescents microscope (Olympus BX63) fitted with filter at Ex/Em=546/575 nm. Morphological images were taken using 50 μm lense and the cells were analysed for cell size width measurements using CellSens Dimension 1,12 software. The surface area of cells from each group (60-80 cells/group) were determined and compared with the AngII-induced cells. Negative control was neither treated nor induced with AngII. The results showed represented analysis from three independent experiments.

ANP Concentration Measurements

After 48 hours the effects of the extracts on levels of ANP were determined and quantified using the rat Atrial natriuretic peptide (ANP) ELISA kit (E-EL-R0017, Elabscience, Biocom Africa) following the same protocol as above using the instructor manual on the cell culture medium. The absorbance of samples and controls were inversely proportional to the concentrations of ANP in the media.

Intracellular ROS Measurements

To measure the reactive oxygen species (ROS) generated by the cells induced with AngII and treated with the extracts, the cells were seeded in 96 wells plates and deprived with serum for 18

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hours. Thereafter the cells were induced with AngII over 2 hours before treated with 50 μg/mL for *Pan cyanecsens* and *P. cubensis* and 25 μg/mL for *P. natalensis* and *P. A+ strain* mushroom extracts and 100 μM losartan for 1 hour. Fluorometric Intracellular ROS assay kit (Green Fluorescence) MAK 143 (Sigma-Adrich) was used according to manual instructions to detect intracellular ROS (especially superoxide and hydroxyl radicals) in live cells with a green fluorescence intensity at λex= 485/20,520/25 nm on AngII-induced cardiomyocytes. The experiment was done in duplicate and repeated three times.

4.3.3. Phytochemical Determination of the Ethanol and Water Extracts

Phytochemical determination of extracts was performed using the gas chromatography-mass spectrometry (GCMS-MS) by the LC-MS (Synapt) facility at the Chemistry Department, University of Pretoria. The water and ethanol extracts of Pan cyanescens, P. cubensis and A+ strain mushrooms were dissolved in methanol (1mg/mL). Chromatograms and presence of compounds in the three extracts were produced. Phytochemistry analysis of *P. natalensis* water and ethanol mushroom extracts were done and the chromatograms and results are published in Nkadimeng et al 2020 [18].

4.3.4. Statistical Analysis

Results are expressed as mean ± standard deviations and statistically significant values were compared using one-way ANOVA analysis of variance using an interactive statistical program (Sigmastat, SPSS version 26, USA) and pairwise multiple comparison procedures using Holm-Sidak method according to [21]. Normality test was done using Shapiro-Wilk and equal variance test using Brown-Forsythe. The p-value of ≤ 0.050 was considered statistically significant. The cold-water, hot-water and ethanol extracts are symbolised in the chapter for *Pan cyanescens* as PC, PH and PE respectively; for *P. cubensis* as GC, GH and GE; for *P. A+ strain* as AC, AH and AE while *P. natalensis* is symbolised as NC, NH and NE respectively and losartan is symbolised with LOS.

5. Conclusions

In conclusion, 70% ethanol, hot-water and cold-water extracts of *Panaeolus cyanescens, Psilocybe nataleases, Psilocybe cubensis,* and *Psilocybe cubensis leucistic A+ strain* mushroom did not exacerbate the AngII-induced hypertrophy and the study revealed for the first time the potential cardio-protective effects of the four mushroom extracts against AngII-induces oxidative stress and hypertrophy in the concentration investigated in the study. Further investigations to support these findings in vivo and also to examine the underlying mechanisms in vivo and in vitro are recommended.

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Data Availability Statement: Data for this research will be obtained from the corresponding author upon request.

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References

1. World Health Organization. 2017. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva.
2. Guzman, G., Allen, J.W. and Gartz, J. 1998. A worldwide geographical distribution of the neurotropica fungi, an analysis and discussion. Annali del Museo civico di storia naturale Giacomo Doria 14, 189–280.
3. Kraehenmann, R., Preller, K.H., Scheidegger, M., Pokomy, T., Bosch, O. G., Seifritz, E. and Vollenweider, F.X. 2015. Psilocybin-induced decrease in amygdala reactivity correlates with enhanced positive mood in healthy volunteers. Biological Psychiatry 78, 572–581.
4. Hasler, F., Grimberg, U., Benz, M.A., Huber, T., and Vollenweider, F.X. 2004. Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. Psychopharmacology 172, 145–156.
5. van Amsterdam, J.G.C., Opperhuizen, A. and van den Brink, W. 2011. Harm potential of magic mushroom: a review. Regulatory Toxicology and Pharmacology 59, 423–429.
6. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. 2018 Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1789–1858.
7. Groenewegen, A., Rutten, F.H., Mosterd, A. and Hoes, A.W. 2020, Epidemiology of heart failure. European journal of heart failure 22, 1342–1356.
8. Curl, C.L., Danes, V.R., Bell, J.R., Raaijmakers, A.J.A., Ip, W.T.K., Chandramouli, C., Harding, T.W., Porrello, E.R., Erickson, J.R. E., Charchar, F.J., et al. 2018. Cardiomyocyte functional etiology in heart failure with preserved ejection fraction is distinctive; A new preclinical model. Journal of the American Heart Association 7, e007451.
9. Shah, A.K., Bhullar, S.K., Elimban, V. and Dhalla, N.S. 2021. Oxidative stress as a mechanism for functional alterations in cardiac hypertrophy and heart failure. Antioxidants 10, 931.
10. Rohini, A., Agrawal, N., Koyani, C.N. and Singh, R. 2010. Molecular targets and regulators of cardiac hypertrophy. Pharmacological Research 61, 269–280.
11. Molkentin, J.D. and Dorn, G.W. 2001. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. Annual Review of Physiology 63, 391–426.
12. Sil, P. and Sen, S. 1997. Angiotensin II and myocyte growth role of fibroblasts. Hypertension 30, 209–216.
13. Wen, H., Gwathmey, J.K. and Xie, L-H. 2012. Oxidative stress-mediated effects of angiotensin II in the cardiovascular system. World Journal of Hypertension 2, 34–44.
14. Liu Q, Wang G, Zhou G, Zhou G, Tan Y, Wang X, Wei W, Liu L, Xue W, Feng W, Cai L. 2009. Angiotensin II-induced p53-dependent cardiac apoptotic cell death: its prevention by metallothionein. Toxicology letters 191, 314–320.
15. Zhang, G-X., Lu, X-M., Kimura, S. and Nishiyama, A. 2007. Role of mitochondria in angiotensin II-induced reactive oxygen species and mitogenactivated protein kinase activation. Cardiovascular Research 79. 204–12.
16. Yoshimura, M., Yasue, H. and Ogawa, H. 2001. Pathophysiological significance and clinical application of ANP and BNP in patients with heart failure. Canadian Journal of Physiology and Pharmacology 79, 730–735.
17. Singh, P., Sharma, R., McElhanon, K., Allen, C.D., Megyesi, J.K., Beneš, H. and Singh, S.P. 2015. Sulforaphane protects the heart from doxorubicin-induced toxicity. Free Radical Biology and Medicine 86, 90–101.
18. Nkadimeng, S.M., Nabatanzi, A., Steinmann, C.M.L. and Eloff, J.N. 2020. Phytochemical, cytotoxicity, antioxidant and anti-inflammatory effects of Psilocybe natalensis magic mushroom. Plants. 9, 127.Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, 2012.
19. Sholeb, M.S. and El-Kadi, A.O.S. 2019. Resveratrol protects against angiotensin II-induced cellular hypertrophy through inhibition of CYP1B1/Mid-Chain hydroxyeicosatetraenoic acid mechanism. The FASEB Journal 2019, 33.
20. Nkadimeng, S.M., Steinmann, C.M.L., Eloff, J.N. 2020. Effects and safety of Psilocybe cubensis and Panaeolus cyanescens magic mushroom extracts on endothelin-1-induced hypertrophy and cell injury in cardiomyocytes. Scientific Reports 10, 22314.
21. Nkadimeng S.M., Steinmann C.M.L., Eloff J.N. 2021. Anti-inflammatory effects of four psilocybin-containing magic mushroom water extracts in vitro on 15-lipoxygenase activity and on lipopolysaccharide-induced cyclooxygenase-2 and inflammatory cytokines in human U937 macrophages. Journal of Inflammation Research. 2021:14, 3729–3738.
22. Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. Anti-inflammatory property of n-Hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology and Drug Design. 2012:80, 434–439.

23. Kumar, P.P., Kumaravel, S. and Latlitha, C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. African Journal of Biochemistry Research 2010:4, 191–195.
24. Korbecki, J. and Bajdak-Rusinek, K. The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms. Inflammation Research. 2019:68, 915–932.
25. Kumaresan, S., Senthilkumar, V., Stephen, A. and Balakumar, B.S. GC-MS analysis and pass-assisted predictions of biological activity spectra of extract of Phomopsis SP. Isolated from Andrographis paniculate. World Journal of Pharmaceutical research. 2015: 4 (1), 1035–1053.
26. Gurudeeban, S., Sathyavani, K. and Ramanathan, T. Bitter apple (Citrulus colocynthis): an overview of chemical composition and biomedical potentials. Asian Journal of Plant Science 20102010, 1–8.
27. Mahmoodreza, M., Forough, K., Hossein, T. and Younes, G. Composition of the essential oil of Rosa damascene mill from South of Iran. Iranian Journal of Psychiatry and Behavioral Sciences. 2010:6, 59–62
28. Ombito, O.J., Matasyoh, C.J. and Vulule, M.J. Chemical composition and larvicidal activity of Zantoxylem gilletii essential oil against Anopheles gambie. African Journal of Biotechnology. 2014:13, 2175–2180.
29. Horiuchi, T. and Tsukamoto, H. Complement-targeted therapy: development of C5- and C5a-targeted inhibition. Inflammation and Regeneration 2016:36.
30. Hema, R., Kumaravel, S. and Alagusundaram, K. GC/MS Determination of Bioactive Components of Murraya koenigii. Journal of American Science 2011:2011, 7.
31. Palariya, D., Singh, A., Dhami, A., Pant, A.K., Kumar, R. and Prakash, O. Phytochemical analysis and screening of antioxidant, antibacterial and anti-inflammatory activity of essential oil of Premna mucronata Roxb. leaves. Trends in Phytochemical Research. 2019:3, 275–286.
32. Asong, J.A., Amoo, S.O., McGaw, L.J., Nkadimeng, S.M., Aremu, A.O. and Otang-Mbeng, W. 2019. Antimicrobial Activity, Antioxidant Potential, Cytotoxicity and Phytochemical Profiling of Four Plants Locally Used against Skin Diseases. Plants. 2019:8, 350.
33. Gideon, V.A. GC-MS analysis of phytochemical components of Pseudoglochidion anamalayanum Gamble: An endangered medicinal tree. Asian Journal of Plant Science. 2015, 5, 36–4.
34. Choulis, N.H. Chapter 49 - Miscellaneous drugs, materials, medical devices, and techniques. Editor(s): J.K. Aronson, Side Effects of Drugs Annual, Elsevier. 2011, 33, 1009–1029.
35. Hernández, M.L., Sicardo, M., Dolores, B.A. and Martínez-Rivas, J.M. The oleic/linoleic Acid ratio in olive (Olea europaea L.) fruit Mesocarp is mainly controlled by OeFAD2–2 and OeFAD2–5 genes together with the different specificity of extraplastidial acyltransferase enzymes. Frontiers in Plant Science. 2021, 12, 345.
36. El Hafidi, M., Pérez, I., Zamora, J., Soto, V., Carvajal-Sandoval, G. and Baños, G. Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology. 2004, 287, R1387-R1393
37. Senol, H, Ozgun-Acar, O., Dağ, A., Eken, A., Guner, H., Aykut, Z.G., Topcu, G., Sen A. Synthesis and Comprehensive in Vivo Activity Profiling of Olean-12-en-28-ol, 3β-Pentacosanoate in Experimental Autoimmune Encephalomyelitis: A Natural Remyelinating and Anti-Inflammatory Agent. Journal of Natural Products. 2023, 86, 103–118. https://doi.org/10.1021/acs.jnatprod.2c00798