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Bioactive chemicals of *Phragmanthera capitata* attenuate depressive-like behaviour in Wistar rats

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Abstract

Objective:
To assess and elucidate the phytochemical constituents of methanol extract of *Phragmanthera capitata* leaves (PCE) and the effect on depressive-like behaviour in using experimental rats.

Methods:
The PCE was obtained using cold marceration with methanol. The crude extract subsequently, was subjected to a comprehensive analysis involving thin-layer chromatography (TLC) and GC-MS techniques to identify its phytochemical constituents. Wistar rats were randomized into 5 groups of 5 rats each. Group I (negative control) received 10 ml/kg of 10% DMSO, Group II (standard) received 30 mg/kg imipramine hydrochloride, and Groups IV-VI (test) received 200, 400 and 800 mg/kg PCE respectively. Unprecedented Chronic Mild Stress (CMS) was induced every day for four consecutive weeks except Group I (control) and body weight was taken regularly. At the end of each stressor session, each rat was returned to the home cage and maintained in standard conditions till the next stress exercise. At the end of induction, the following tests were performed: Sucrose Preference Test (SPT) to assess anhedonia, Tail suspension test (TST) and Forced swimming test (FST) to assess behavioural despair.

Results:
The phytochemical studies and analyses indicated the presence of vinylgualacol, acetophenone, syringol, trans-isoeugenol, caryophyllene oxide, 2-aminoacetanilide, and hexahydrofarnesyl acetone. There were significant ($p \leq 0.05$) increase in body weight, increase in sucrose preference index and decrease immobility time as compared to negative control.

Conclusion:
Phytochemicals of *P. capitata* methanol leaves extract attenuate depressive-like behavior in Wistar rats.

Keywords: *Phragmanthera capitata;* phytochemicals; depression; leaf extract; imipramine

1. Introduction

Depression is a life-threatening mental disorder characterized by low mood, low self-esteem and loss of pleasure with occasional delusions or hallucinations [1, 2]. Diagnosis is based on the person's reported experiences and a mental status examination and there is no laboratory test for the disorder. However, testing may be done to rule out physical conditions that can cause similar symptoms [3]. The most

common time of onset of depression is in a person's 20s, with females affected about twice as often as males [4, 5]. The course of the disorder varies widely from one episode lasting months to a lifelong disorder with recurrent major depressive episodes[6].

Treatment of depression is typically with psychotherapeutic and antidepressant medications[7]. Medications appear to be effective, but the effect may only be significant in the most severely depressed [8,9]. Hospitalization, which may be involuntary, may be necessary in cases with associated self-neglect or a significant risk of harm to self or others. Electroconvulsive therapy may be considered if other measures are not effective [2].

Nevertheless, it is estimated worldwide that 3.8% of the population is affected with depression, including 5.0% among adults and 5.7% among adults older than 60 years [10]. Approximately 280 million people in the world have depression [10]. At its worst, depression can lead to suicide. Over 700 000 people die due to suicide every year. Suicide is the fourth leading cause of death in 15-29-year-olds [11,10].

Newer orthodox medications for treating depression are grouped into three classes: selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, and norepinephrine-dopamine reuptake inhibitor. While these drugs offer superior tolerability and safety over older medications such as the tricyclic antidepressants, there remains no universally effective pharmacologic treatment for depression [12]. Besides more than 75% of people in low and middle income countries receive no treatment mainly because of high cost or non-availability of medications [2].

Phragmanthera capitata is mistletoe of Loranthaceae Family. It has been found to have anti-diarrheal properties; anti-pyretic and analgesic potentials; steroid genetic and spermatogenetic activities; exploratory and anxiety potentials; and haematopoietic activities [13- 17]. This study represents major advances in understanding the phytochemical constituents of *Phragmanthera capitata* and highlights the associated antidepressant effect.

2. Materials and methods

2.1 Plant material

Phragmanthera capitata leaves were harvested and processed as earlier described [13]. Briefly, leaves of *P. capitata* were collected in the month of March 2023 from the botanical garden of the University of Calabar and authenticated in the Department of Botany with a voucher specimen number UC/23/016. The leaves air dried at room temperature (26^0 c) were pulverized using electric blender, into powder. The powdered leaf (200 g) was extracted by cold maceration (48 h) with methanol; then filtered to obtain the methanol extract. At reduced pressure using a rotary evaporator, the extract was concentrated and further oven-dried. The extract was subjected to analysis of phytochemicals using

standard method [18, 19]. The comprehensive isolation and characterization of the phytocompounds were performed using Column Chromatography, and GC-MS (thermo Scientific Co; as described by the manufacturer).

2.2 Animals

Wistar rats of both sexes and having between 150-200 g were purchased from University of Calabar, Department of Pharmacology Animal House Unit, and kept in polyvinyl cages with soft wood shavings as bedding. Standard laboratory conditions observed were temperature about 26^0C, relative humidity about 50%, as well as 12 hour light: dark photoperiod. For animal nutrition, standard Agro Feeds, Calabar was used and water *ad libitum.* The protocols for the study were approved by the Faculty of Basic Medical Sciences Ethics Committees UNICAL, Nigeria in conjunction with OECD guidelines[20].

2.3 Methods

2.3.1 Qualitative and quantitative analysis of phytocompounds

The phytochemical identification and quantification was performed on a GC-MS (Thermo Scientific Co.) system. The experimental conditions of GC-MS system were as described by the manufacturer.

2.3.2 Experimental procedure

CMS-induced depressive-like behavior

Healthy rats of 150-200 g weight were put at random into 6 groups of 4 rats. Group I (positive control) and Group II (negative control) were administered 10 ml/kg of 10% DMSO (Sigma Aldrich, USA), Group III (standard) received 30 mg/kg imipramine hydrochloride (Sigma Aldrich, USA), and Groups IV-VI (test) were administered 200, 400 and 800 mg/kg PCE respectively. Extract and drug were dissolved in 10% DMSO and administered per oral. CMS was induced every day for four consecutive weeks except Group I (positive control). The stressors used included water deprivation for 24 h, food withdrawal for 24 h, reverse light/dark for 24 h, a cold swim at 4°C for 5 min, thermal stimulus in an oven at 45°C for 5 min, tilting of cage at 45° for 24 h, pairing for 5 h. At the end of each stressor session, each rat was returned to the home cage and maintained in standard conditions till the next stress exercise[21].

2.3.3 Weight measurement

Weights of rats were taken at the end of each week using a triple beam balance (Nimble Gymnastic Equipment, USA).

2.3.4 Sucrose Preference Test (SPT)

All rats were habituated 72 hours before the test with 1% solution of sucrose: 2 bottles comprising of 1% solution of sucrose (w/v) were put in each cage and after 24 hours, one bottle was substituted with

a bottle containing tap water for the next 24 hours. This was to give the animals enough time to adapt to sucrose solution. Then animals were food and water deprived for another 24 hours. Then SPT was conducted where each rat was given 2 bottles containing 1% solution of sucrose (w/v) as well as tap water to freely drink for 12 hours. In other to avoid position effects, 6 hours later, 2 bottles were exchanged and twelve hours later, consumption of tap water as well as sucrose solution were recorded. Sucrose preference index (SPI) was calculated according to Du *et al*. [22].

$$\text{SPI (\%)} = \frac{Sucrosesolutionconsuption}{Sucrosesolutionconsuption + Tapwaterconsumption} X\ 100$$

2.3.5 Tail suspension test (TST)

Immediately after SPT, each rat was suspended on an edge 50 cm above the floor level by adhesive tape about 1 cm from the tail tip for a duration of 6 minutes. Duration of immobility was recorded by a stop watch. Rats were considered immobile when they showed passive and complete motionlessness [23].

2.3.6 Forced swimming test (FST)

Animals were gently introduced into an open cylindrical container of 2.5 L total volume of water; height of 20 cm and diameter of 14 cm contained 10 cm of clean water at 25^{o}C. Immobility duration was recorded for six minutes when a rat stopped struggling and remaining floating motionless while making only movements necessary to keep its head above water [22].

2.4 Statistical analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple range test for post hoc. The values were expressed as mean ± SEM. The statistical difference was considered significant at $p \leq 0.05$.

3. RESULTS

3.1 Analysis of phytocompounds

The comprehensive analyses involving column chromatography and GC-MS showed the presence of Vinylgualacol, acetophenone, syringol, trans-isoeugenol, caryophyllene oxide, 2-aminoacetanilide, and hexahydrofarnesyl acetone(Table 3, and Figure 1) with reference to NIST II Library research report TIC (Tables 1 and 2).

Extract treatment of rats induced with unprecedented chronic mild stress (CMS) for a month, showed a significant ($p < 0.05$) gain in weight for both PCE and imipramine treated groups when compared to the negative control group (Figure 2).

The anhedonia induced by CMS was revered in rats treated with PCE and imipramine (Figure 3).

In tail suspension test (TST) immobility duration was significantly ($p < 0.05$) reduced by groups treated with PCE and imipramine (Figure 4). The same result was applicable to forced swimming test (FST) (Figure 5).

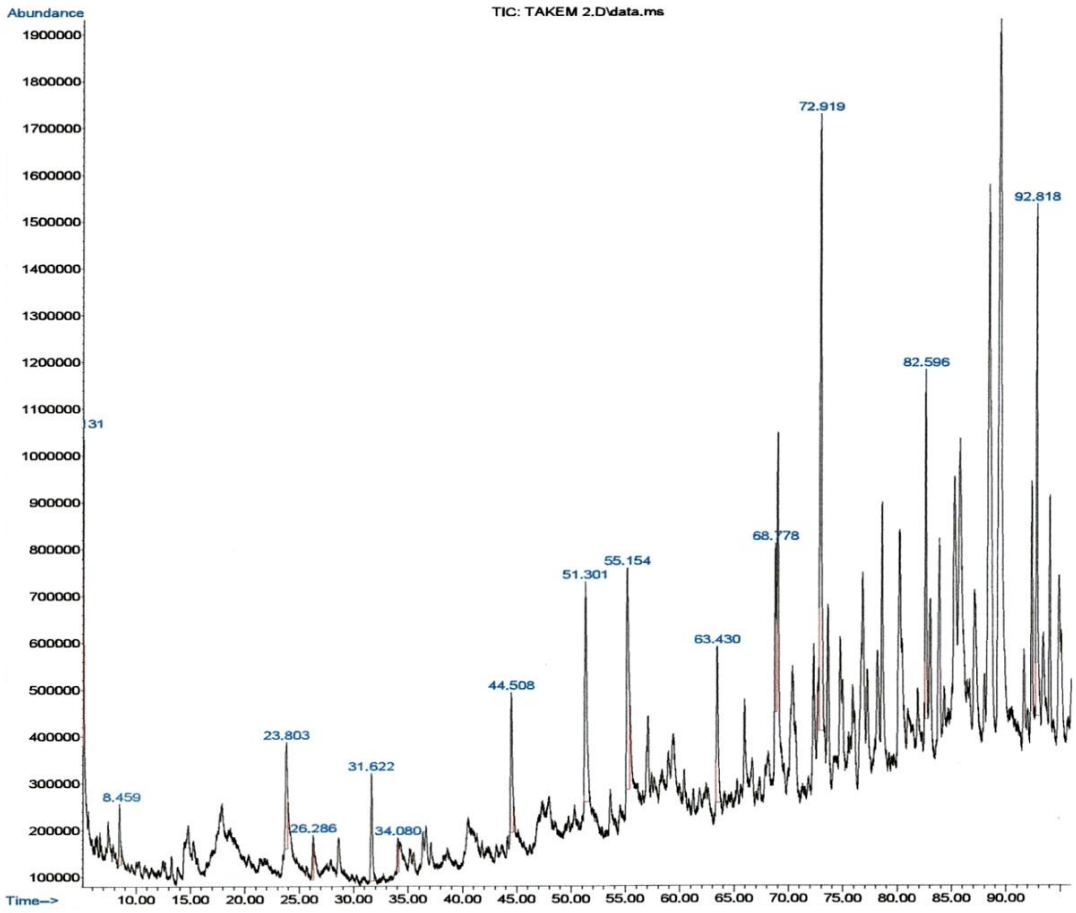

Fig. 1: Total ion chromatogram (TIC) of PCE

TABLE 1: Phytocompounds identified in PCE by GC-MS NIST 11 Library Research Report TIC

Pk#	RT (min)	Area%	Library/ID	Ref#	CAS#	Qual
1	5.131	3.82	trans-4,4-Dimethyl-2-hexene	6754	019550-83-5	80
			3-Hexene, 3,4-dimethyl-, (Z)-	6774	019550-87-9	80
2	8.459	1.81	2(5H)-Furanone, 5-methyl-	3103	000591-11-7	72
			Diethylcyanamide	3055	000617-83-4	64
			2(5H)-Furanone, 5-methyl-	3105	000591-11-7	64
3	23.803	4.79	Phenol, 2-methoxy-	10417	000090-05-1	91
			Phenol, 2-methoxy-	10420	000090-05-1	91
			Phenol, 2-methoxy-	10419	000090-05-1	90
4	26.286	0.97	Cyclopentene	431	000142-29-0	58
			Cyclopentene	426	000142-29-0	53
			Cyclopentene	429	000142-29-0	53
5	31.622	4.03	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	26054	001125-21-9	95
			2,6,6-Trimethyl-2-cyclohexene-1,4-dione	26060	001125-21-9	91
			2,6,6-Trimethyl-2-cyclohexene-1,4-dione	26059	001125-21-9	91
6	34.080	0.74	Acetic acid, 4-methyl-2-oxotetrahy dropyran-4-yl ester	40177	083191-98-4	64
			5-Cycloheptene-1,4-dione, 2,2,5-trimethyl-	34896	058705-37-6	47
			Dodecanoic acid, hex-3-enyl ester	129370	1000159-94-3	38
7	44.508	5.41	trans,trans-4-methyl-3-oxabicyclo[4.4.0]decane	26885	1000216-87-9	38
			Pyridine, 3-(ethylthio)-5-Amino-2-methoxyphenol	17818	026891-59-8	38
				17788	001687-53-2	37
8	51.301	9.92	2-Methoxy-4-vinylphenol	24419	007786-61-0	90
			Ethanone, 1-(2-hydroxy-5-methylphe nyl)-	24526	001450-72-2	80
			Benzene, [2-(methylthio)ethenyl]- (E)-	24560	015436-06-3	64
9	55.154	10.60	Phenol, 2,6-dimethoxy-	27407	000091-10-1	97
			Phenol, 2,6-dimethoxy-	27403	000091-10-1	95
			Phenol, 2,6-dimethoxy-	27406	000091-10-1	91
10	63.430	5.96	trans-Isoeugenol	33250	005932-68-3	97
			Phenol, 2-methoxy-4-(1-propenyl)- (Z)-	33448	005912-86-7	97
			trans-Isoeugenol	33252	005932-68-3	96
11	68.778	3.92	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	45525	015356-74-8	94
			2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	45536	017092-92-1	94
			2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	45539	017092-92-1	45
12	72.919	21.95	Caryophyllene oxide	77539	001139-30-6	95
			Caryophyllene oxide	77538	001139-30-6	89
			Caryophyllene oxide	77536	001139-30-6	62
13	82.596	10.45	2-Aminoacetanilide	24283	034801-09-7	30
			2-Acetyl-1-phenylhydrazine	24307	000114-83-0	25
			Acetamide, N-(3-aminophenyl)-	24315	000102-28-3	25
14	92.818	15.62	2-Pentadecanone, 6,10,14-trimethyl	117630	000502-69-2	91
			2-Pentadecanone, 6,10,14-trimethyl	117629	000502-69-2	80
			2-Pentadecanone, 6,10,14-trimethyl	117627	000502-69-2	59

TABLE 2: Phytocompounds identified in PCE by GC-MS Area Percent Report TIC.

Peak	RT (min)	First scan	Max scan	Last scan	PK Ty	Peak height (mm)	Corr area (mm)	Corr % max	% of total area
1	5.131	3	7	26	rVB2	679078	2396881	17.40	3.818
2	8.459	525	539	571	rBV3	130529	1138297	8.26	1.813
3	23.803	2964	2992	3018	rBV4	227286	3005234	21.81	4.788
4	26.286	3355	3389	3393	rBV4	95803	608854	4.42	0.970
5	31.622	4214	4242	4281	rBV4	230054	2532018	18.38	4.034
6	34.080	4620	4635	4643	rBV5	73686	464435	3.37	0.740
7	44.508	6278	6302	6335	rBV4	299234	3398813	24.67	5.415
8	51.301	7367	7388	7454	rVB3	470736	6227894	45.20	9.922
9	55.154	7983	8004	8043	rBV3	473797	6652992	48.29	10.599
10	63.430	9312	9327	9379	rVB6	333896	3740533	27.15	5.959
11	68.778	10169	10182	10195	rBV6	362117	2460547	17.86	3.920
12	72.919	10827	10844	10899	rVB6	1320334	13778168	100.00	21.950
13	82.596	12375	12391	12427	rVB6	748123	6559432	47.61	10.450
14	92.818	14007	14025	14081	rVB5	1094811	9806539	71.17	15.623

TABLE 3: Characterization of most abundant compounds found in PCE

CAS number	Compound name	Molecular weight (g/mol)	Molecular formula	Peak height (mm)	% Total area	Use
7786-61-0	Vinylguaiacol	150.1745	$C_9H_{10}O_2$	47.0736	9.922	Beer flavour
1450-72-2	Acetophenone	150.1745	$C_9H_{10}O_2$			Synthesis of procyclidine
91-10-1	Syringol	154.1632	$C_8H_{10}O_3$	47.3797	10.599	Preparing smoking food
5932-68-3	Trans-Isoeugenol	164.2011	$C_{10}H_{12}O_2$	33.3896	10.599	Fragrance
1139-30-6	Caryophyllene oxide	220.3505	$C_{15}H_{24}O$	13.20334	21.950	Neuroprotective, Anxiolytic and antidepressant [27]
34801-09-7	2-Aminoacetanilide	150.1778	$C_8H_{10}N_2O$	74.8123	10.450	Chemical plant
502-69-2	Hexahydrofarnesyl acetone	268.4778	$C_{18}H_{36}O$	109.4811	15.623	Flavour and fragrance

3.2 Weight measurement

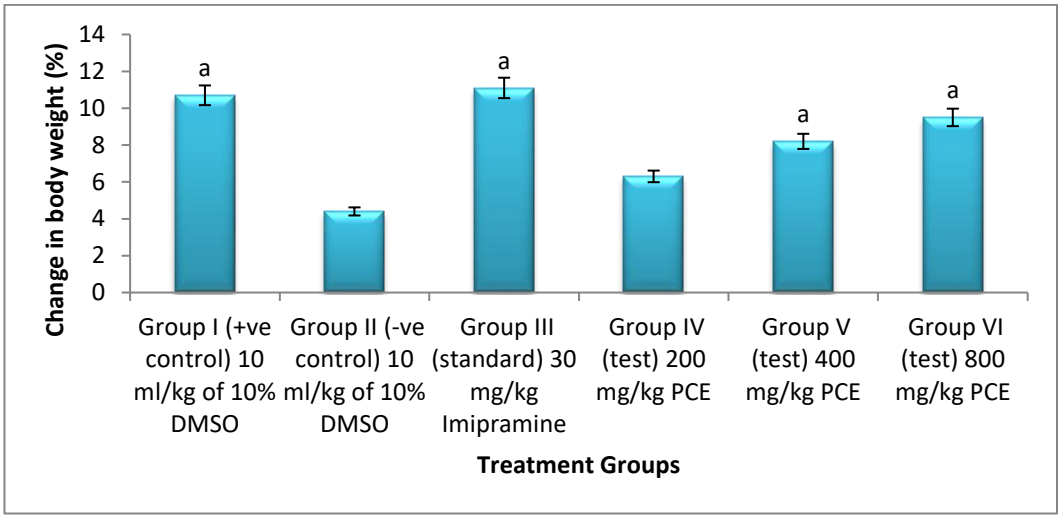

Fig. 2: Effect of PCE on body weight.Each value is mean ± SEM for 4 rats. ($^{a}P \leq 0.05$)

3.3 Sucrose Preference Test

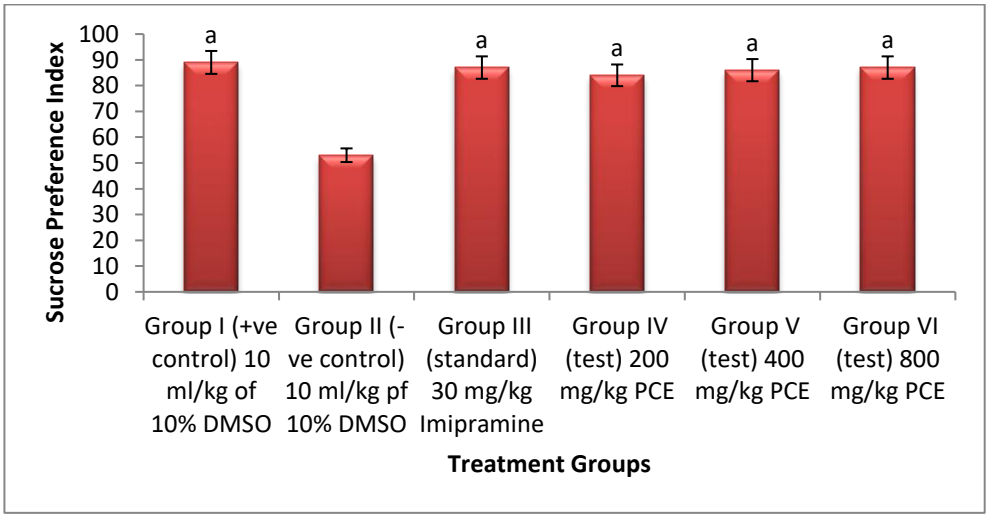

Fig. 3: Effect of PCE on sucrose preference test.Each value is mean ± SEM for 4 rats. ($^{a}P \leq 0.05$)

Tail Suspension Test

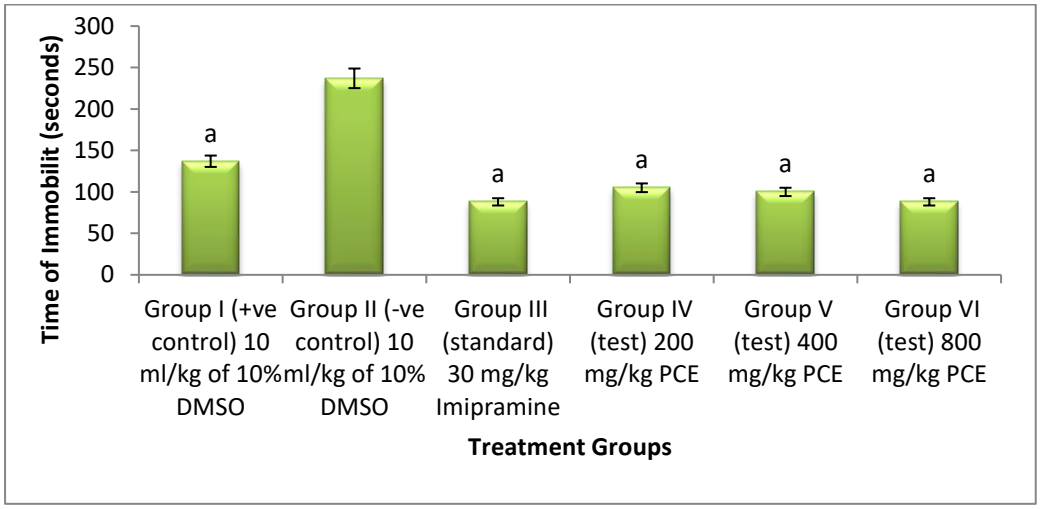

Fig. 4: Effect of PCE on immobility time Each value is mean ± SEM for 4 rats. ($^{a}P \leq 0.05$)

3.5 Forced Swimming Test

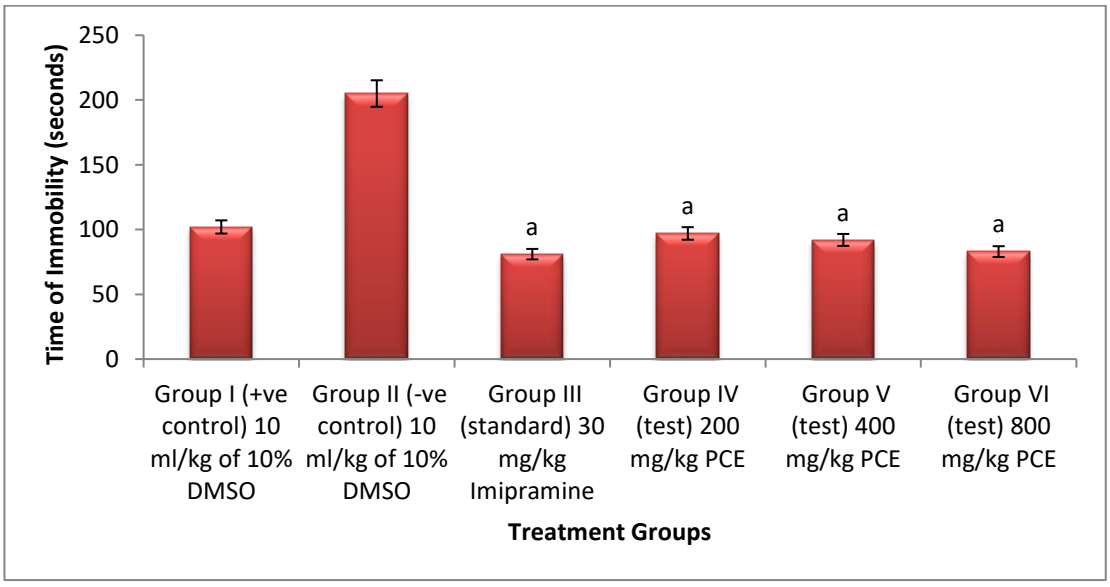

Fig. 5:Effect of PCE on Forced Swimming Test. Each value is mean ± SEM for 4 rats. ($^{a}P \leq 0.05$)

4. Discussion

The model of chronic mild stress (CMS) is seen as maybe the most valid animal model of depressive behaviour which is aimed to mimic chronic depressive-like state. This state is believed to develop slowly as time passes in response to stressors and it does provide much more natural induction. This

model is being documented as resulting in long lasting neurochemical, behavioural, neuroendocrinological and neuroimmune changes. These changes mimic reward functions that include a reduction intracranial self-stimulation, a reduction in reflecting anhedonia that is known to be reversed by chronic but not acute antidepressants. In this research work, the endophenotypic parameters of animal model of depression that were evaluated were weight changes representing changes in appetite, sucrose preference test - representing anhedonia, tail suspension test and forced swimming -representing behavioural despair.

Imipramine was used as standard drug. Imipramine is a tricyclic antidepressant drug that does work in antagonizing neuronal reuptake of noradrenaline and serotonin neurotransmitters. Imipramine binds to sodium-relying noradrenaline transporter and sodium-relying serotonin transporter to reduce reuptake of these neurotransmitter by neurons[25]. Lack of stimulation of post-synaptic serotonergic and noradrenergic neurons has been shown to be the cause of depression. Therefore, slowing the reuptake of these neurotransmitters augment their synaptic cleft concentration and produce knock-on effects in protein kinase signalling circuits known in contributing to changes in neurotransmission and brain physiology resulting in relieve of symptoms of depression[26].

In weight changes, the two hormones called leptin and ghrelin are responsible for controlling metabolism and hunger. While leptin does block appetite and increase energy expenditure, ghrelin does increase appetite and reduce energy expenditure[27]. In this research work, treatment of rats induced with unprecedented CMS for a month showed a significant gain in weight for both imipramine and PCE treated groups when compared to negative control group. This may suggest that there was potentiation of ghrelin activity by PCE. Ghrelin binds on growth hormone secretagogue receptor in the brain which is G protein-coupled receptor thereby regulating body weight and energy homeostasis [28].Therefore, some components of PCE could have a synergic activity at ghrelin receptor.

In sucrose preference test representing anhedonia, the sweet taste of sucrose is a rewarding stimulus for both rodents and primates. Central dopamine has been extensively studied and known to be critical in potentiating subjective pleasure linked to positive rewards. The mesolimbic dopaminergic system has been identified as playing a key role in regulating reward-related behaviour such as food and sucrose [29, 30]. In this present research, the anhedonia induced by CMS was reversed in rats treated with imipramine and PCE. Imipramine is also known to inhibit D_2 subtype of dopamine receptor which is Gi subtype of G protein coupled receptor [31]. This blockage causes dopamine to bind more to Gs subtype of G protein thereby enhancing dopaminergic reward-related behaviour. In the same vein, PCE is suggested to enhance mesolimbic dopaminergic reward-related behaviour since anhedonia was reversed by both groups. The ability of a plant extract to stimulate the release or activity of a

neurotransmitter has been demonstrated earlier, particularly in the case of *Thunbergia laurifolia*, where report showed that *T. laurifolia* extract was able to stimulate rat dopamine release from the *nucleus accumbens* of rats, a part of what is known as the reward system[32]. Another study by Hosseinali reported that aqueous extract of *Crocus sativus* increased brain dopamine and glutamate concentrations in rats[33]. All these findings reveal plant extracts may have the capacity to affect reward behaviour of rats by either potentiating it through increased dopaminergic excitation by blocking the Gi-mediated modulation or through increase production of dopamine.

Immobility in tail suspension test can be interpreted as strategy of passive stress-coping or depressive-like behaviour or better still behavioural despair. In this research work, immobility duration was significantly reduced by groups treated with imipramine and PCE. This suggests that PCE mimicks imipramine in binding to sodium-relying noradrenaline transporter and sodium-relying serotonin transporter to reduce reuptake of these neurotransmitter by neurons. However, the precise mechanisms of action are still yet to be elucidated; hence it is open for further investigations.

In conclusion, the research findings suggest that methanol leaves extract of *P. capitata* phytochemicals attenuate depressant-like behaviour in Wistar rats.

Conflict of interest statement

The Authors declare that there is no conflict of interest.

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Author's contributions

LPT conceptualized and supervised the work, involved in literature search and writing of manuscript. JLA did statistical analysis and manuscript editing. SCO participated in data acquisition, writing and editing of the manuscript. All authors read and approved the manuscript for submission.

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