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Methanol stem extract of *Parquetina nigrescens* (Asclepiadaceae) possesses memory-enhancing potential in acute mice models of cognition

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ABSTRACT

Background & Aim: *Parquetina nigrescens* is an important plant in the Southwestern part of Nigeria used as memory enhancer. This study aimed to investigate the memory enhancing effect of the methanol stem extract of *P. nigrescens* (MSEPN).

Experimental: Phytochemical screening was carried out on the extract and the oral median lethal dose (LD50) determined using the OECD 425 limit test. The effects of MPN (250, 500 and 1000 mg/kg) on learning and memory was evaluated in elevated plus maze (EPM), Barnes maze (BM) and novel object recognition test (NORT). The locomotive activity was studied using the open field test (OFT). The antiamnesic effect of the extract was assessed in EPM.

Results: The phytochemical constituents in MSEPN were tannins, saponins, phenolics, carbohydrates and unsaturated sterols. The LD50 was ≥ 5000mg/kg. Piracetam and the extract significantly ($p \leq 0.05$) decreased transfer latencies (TL) on day 1 and 2 in EPM. In the BM, the escape latencies and escape errors were decreased significantly ($p \leq 0.05$, $p \leq 0.01$) at 250 and 1000 mg/kg, respectively. The time spent in target quadrant (TSQ) was significantly ($p \leq 0.01$) increased at 250 and 500 mg/kg of extract. Piracetam significantly decreased escape latency ($p \leq 0.05$), escape errors ($p \leq 0.01$) and increased TSQ. Discrimination index in the NORT was significantly ($p \leq 0.01$) increased. The extract did not significantly alter the number of square cross (NSC) and rearing (NR) but significantly ($p \leq 0.05$) decreased TL increased by diazepam on day 1. Piracetam increased NSC and NR and significantly ($p \leq 0.01$, $p \leq 0.05$) decreased TL on day 1 and 2 in EPM.

Recommended applications/industries: The memory enhancing potential of MSEPN can be applied in the treatment of amnesia.

1. Introduction

Memory is the ability to record, retain information and retrieve the same when needed while learning is a process of acquiring new information about occurring events (Markowitsch, 1988; Devi *et al.*, 2011). Enhancing memory implies an improvement in the processing of information systems and the extension of its main capacities (Hideyuki *et al.*, 2000; Shivani *et al.*, 2016). Therefore, memory enhancers are drugs or substances capable of improving the powers of acquisition, retention and retrieval of information. Age, stress, anxiety and some pharmacological agents are

known to cause learning and memory impairment. Therefore, animal models have been helpful for screening agents capable of enhancing memory and understanding the processes that impair cognitive function. For example, exteroceptive models such as elevated plus maze; Barnes maze; and novel object recognition test assess anxiety-related learning and memory; spatial learning and memory; and recognition memory (in the absence of stress) respectively while the interoceptive models involve use of pharmacological agent e.g diazepam to impair learning and memory.

There is an increasing demand for memory enhancers among healthy individuals and those with disease conditions like schizophrenia, depression, Alzheimer's disease etc associated with memory impairment (Rathhee *et al.*, 2008; Leslie *et al*., 2015). Drugs like amphetamine, methylphenidate and caffeine commonly used to enhance learning and memory are not only stimulants but also have addictive potential and therefore become a major public health problem (Fond *et al.*, 2015). Current drugs such as donepezil, rivastigmine, galantamine e.t.c are of limited benefit in restoring normal cognitive function in patients with memory impairment since they only provide symptomatic relief and do not halt the progression of the disease (Deepika *et al*., 2010). Hence, there is need for the search of safer and effective memory enhancers.

The traditional system of medicine is replete with the use of medicinal plants known to enhance memory in different part of the world (Yu *et al.*, 2005; Adsersen *et al*., 2006). For example, the Indian system of medicine is rich of memory enhancing medicinal plants, which are today popular due to their proven efficacy (Rathhee *et al.*, 2008; Balkrishna and Misra, 2017). And in Nigeria, a number of medicinal plants are used to enhance memory. One of such medicinal plant that has enjoyed a wide patronage among traditional medicine practitioners in South-Western part of Nigeria is *P. nigrescens* (Elufioye *et al.*, 2012). *P. nigrescens* is a climbing, twinning or sometimes erect, softly woody usually found in the forest and growing on ant hills across Africa region (Burkill, 1985, Ayinde *et al.*, 2015). Different parts of the plant are used as medicine for the treatment of snakebite, wounds, piles, diabetes, as aphrodisiac, etc (Borokini *et al*., 2013, Gbadamosi, 2015). The roasted stems and roots are powdered then mixed with pap and taken as memory enhancer (Elufioye *et al.*, 2012). Therefore, this study was undertaken to investigate the memory enhancing effect of methanol stem extract of *P. nigrescens*.

2. Materials and Methods

2.1. Drugs and chemicals

Piracetam (Nootropil^{R} UCB Pharma Limited), gum acacia and methanol (Zayo-Sigma Chemicals ltd), diazepam ((Valium^{R}, Roche).

2.2. Animals

Swiss Albino mice of both sexes weighing 18-25 g were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) Zaria. The mice were maintained on standard laboratory animal feed and allowed access to water. Mice were acclimatised for five days and randomly selected into various treatment groups before experimentation. Institutional Animal Ethics Committee's approval was obtained (ABUCAUC/2017/023). Experiment was carried out between 9:00 and 18:00 hours of the day.

2.3. Plant Collection and Preparation of Extract

The plant material comprising the leaves and stems of *P. nigrescens* was collected in the bush within Zaria metropolis of Kaduna, Nigeria in the month of June, 2016. The freshly harvested plant material was identified by Mallam Umar Gallah of the Herbarium section of the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna. A voucher specimen (01624) was deposited in the Herbarium unit of NARICT.

The stems were collected, washed and shade-dried for three weeks after which it was grounded to coarse powder using mortar and pestle. The powdered sample of 1000 g was extracted with 10 litres of 70 % methanol by cold maceration with intermittent shaking for one week. The macerated mixture was filtered and the filtrate concentrated using a rotary evaporator. The concentrate was dried over a water bath at a temperature of 40^{o}C to obtain a brown residue subsequently referred to as the Methanol Stem Extract of *Parquetina nigrescens*. This was stored in a desiccator until required for use.

2.4. Phytochemical Analysis

Phytochemical analysis was carried out on the methanol extract according to the method described by Evans (1996).

2.5. Acute Toxicity Study

The oral Median Lethal Dose (LD_{50}) of the extract in mice was estimated using the (OECD) 425 guideline limit test (OECD, 2001).

2.6. Behavioural Studies

2.6.1. Elevated plus maze

The method previously described by Parle (2003) and Dhingra (2004) was slightly modified and adopted to assess learning and memory. The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze elevated to a height of 45 cm from the floor.

Briefly, 30 mice were randomly divided into five groups of six animals and treated with 1 ml/100 g of 1 % gum acacia (group 1), 400 mg/kg piracetam (group 2), 250, 500 and 1000 mg/kg extract (group 3, 4 and 5 respectively). 1 % gum acacia was used to suspend the extract. One, two and twenty four hours post treatment, each mouse was placed at the end of an open arm, facing away from the central platform and the transfer latency (TL) taken for training, learning and memory respectively. Transfer latency was defined as the time taken by the animal to move from the end of the open arm into one of the covered arms with all its four legs. Sixty seconds maximum TL was assigned for each animal and while in any of the closed arm a maximum of 20 seconds was allowed for the animal to explore the maze and thereafter returned to its home cage. Animals that did not enter into either one of the covered arm within 60 seconds were gently pushed in and TL assigned 60 seconds. The maze was cleaned after each trial with 70 % ethanol to remove any olfactory cue.

2.6.2. Barnes maze

The procedure for testing spatial learning and memory was followed as per the method developed by Barnes (1979) with slight modification. The maze consisted of a white-painted circular wooden platform (95 cm in diameter) with 40 equally spaced holes (5 cm in diameter and 5 cm between holes) along the perimeter in which a recessed goal box (10 x 20 cm) is located underneath one of the holes and the maze elevated to 105 cm above the floor. A circular start box (15 cm in height and 20 cm in diameter) usually put at the centre of the maze containing the animal for 10 seconds before each trial was used. The room was configured with posters for spatial navigation.

Briefly, mice were trained for two days to locate and enter a goal box with the aid of a fan (an aversive stimulus). Each mouse was placed in the start box located at the centre of the maze for 10 seconds which was thereafter lifted away. The fan was switched on and the mouse was allowed to explore for 2 min to locate and enter the target box. Upon entering the goal box, the fan was switched off and animals were allowed to stay undisturbed for 20 seconds before returning to the home cage. This testing process was repeated for 3 additional trials with an inter-trial interval of 15 minutes.

On Day 3 (acquisition trial), 30 mice were randomly selected into five groups of six animals and treated with 1 ml/100 g of 1 % gum acacia (group 1), 400 mg/kg piracetam (group 2), 250, 500 and 1000 mg/kg extract (group 3, 4 and 5 respectively) per oral. 1 hour after drug administration each animal underwent four training as previously described. Primary latency and errors, total latency and errors were taken for each animal as indices of learning. Primary latency defined as the time taken to locate the target hole but no entering. Primary error is the number of head dip before first locating the escape hole. Total latency is the time taken to locate and enter the goal box. A total error is the total number of head dip before entering the goal box. Animals that failed to find the goal location within the 2 minutes trial were gently guided into it, and allowed to remain for 20 seconds then assigned a total latency of 120 s. The maze was cleaned after each trial with 70 % ethanol to eliminate olfactory cue. On day 4 (probe trial), the goal box was removed and the maze platform divided into four quadrants viz: target, opposite, positive and negative quadrant each containing ten holes. Memory retention was assessed by quantifying the time spent in target quadrant under the coverage of a video camera placed over the maze. The maze was cleaned after each trial with 70 % ethanol to remove any olfactory cue.

2.6.4. Novel object recognition test (NORT)

The method described by Ennaceur (2010) and modified by Gaskin *et al.,* (2010) was used to assess recognition memory. The apparatus consists of a plexiglass box of 40 cm x 40 cm x 40 cm in dimension. The NORT consist of three phases: the habituation, familiarization and testing phase. On day 1, mice were habituated to the open arena without object for 2 minutes and returned to the home cage. On day 2, two identical objects were introduced into the arena of the apparatus 20 cm apart and 5 cm away from the walls of the apparatus. Each mouse was allowed to explore the identical objects for 10 minutes and returned to the home cage. On day 3 (testing day), one of the objects was replaced with another (a novel object) of different size and colour. 30 mice were randomly selected into five groups of six animals and treated with 1 ml/100 g of 1 % gum acacia (group 1), 400 mg/kg piracetam (group 2), 250, 500 and 1000 mg/kg extract (group 3, 4 and 5 respectively) per oral. 1 hour after drug administration mice were introduced into the arena and allowed to explore for 5 minutes. The behaviour of mice was recorded with the aid of a camera. The time for novel and familiar object exploration were taken and the discrimination index of memory calculated (difference between novel and familiar object exploration). Object exploration is defined as the time taken for the animal's orientation towards the object with the snout, sniffing and touching the object. While climbing or sitting on the object was not considered as exploration (Aggleton *et al.,* 2010). The apparatus was cleaned after each trial with 70 % ethanol to remove any olfactory cue.

2.6.5. Open field test

The method described by Kalueff *et al.,* (2006) was adopted for the assessment of spontaneous locomotor (horizontal) and exploratory (vertical) activity. The apparatus consists of plywood (72 x 72 x 36 x 36 cm). One of the walls is a clean transparent plexiglas for visibility. The base was divided into 16 squares (18 x 18 cm) with blue marker and covered with transparent plexiglas (Brown *et al.,* 1999).

Mice were randomly selected into five groups of six animals each. Groups 1, 2, 3, 4 and 5 received 1 ml/100 g 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per oral respectively. One hour post drug administration, each mouse was placed individually at the corner of the arena and its behaviour monitored for 5 minutes with a video camera hung 1.5 meter above the apparatus. The number of square cross and rearing was recorded for each animal. The apparatus was cleaned in-between observations with 70% methanol and allowed to dry to remove any olfactory cue.

2.6.6. Diazepam induced amnesia

Mice were randomly selected into six groups of six animals each. Groups 1, 2, 3, 4, 5 and 6 received 1 ml / 100 g 1% gum acacia, 1 ml /100 g 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg of the extract per oral respectively. Amnesia was induced with diazepam (dose: 0.7 mg/kg intraperitoneally) in animals of groups 2, 3, 4, 5 and 6, 30 minutes after oral drug administration to the respective treatment group. Transfer latency (TL) was recorded 30 minutes (training), 1 hour (learning) and 24 hours (memory) post diazepam administration in the elevated plus maze as previously described.

2.7. Statistical Analysis

The data were expressed as Mean ± Standard Error of Mean and analysed using One-Way ANOVA. Any significant difference was assessed by Dunnett's post-hoc t-test for multiple comparisons. $p \leq 0.05$ was considered to be statistically significant.

3. Results and discussion

The increasing demand for memory enhancers among healthy individuals, those with cognitive impairment and the inefficacy of the existing agents in reversing neurodegeneration that impact on cognitive function have led to the continuous search for more effective and safer agents. A number of medicinal plants used as memory enhancers among traditional medicine practitioners are yet to be validated. The plant *P. nigrescens* is used as memory enhancer among the Yorubas of Nigeria (Elufioye *et al.,* 2012). In this study, the effect of *P. nigrescens* on learning and memory was investigated using different cognitive animal models assessing a specific kind of memory.

In the elevated plus maze, the extract produced a dose-dependent decrease in transfer latency on day 2 at all doses which was only significant at 500 and 1000

mg/kg ($p \leq 0.05$ and $p \leq 0.01$respectively). The extract decreased transfer latency on day 1 only at 250 and 1000 mg/kg ($p \leq 0.05$). There was no decrease in transfer latency on day 1 at 500 mg/kg. Piracetam significantly decreased the transfer latency on day 1 and 2 ($p < 0.01$ and $p < 0.05$, respectively) (Figure 1).

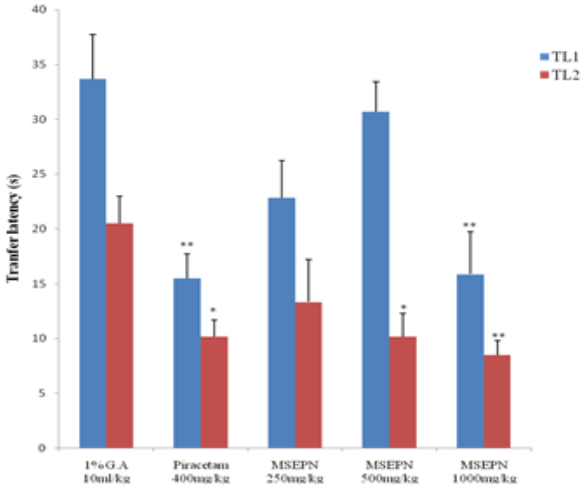

Figure 1: Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency of mice in an elevated plus maze. Data presented as mean ± standard error of mean (n=6); *$p \leq 0.05$; **$p \leq 0.01$ as compared to 1 % G.A group; TL1: learning; TL2: memory; G.A: gum acacia; S: seconds; MSEPN: methanol stem extract of *P. nigrescens*

The decreased in transfer latency on day 1 and 2 is an indication of improvement in learning and memory respectively. Elevated plus maze is a paradigm used to asses cognitive function and anxiety related behavioural studies (Silveira *et al.*, 1993; Gonzalez and File, 1997). Anxiety is thought to disrupt cognitive function by taking reins of sensory, perceptual and attentional processes in certain areas of the brain therefore, threatening information are preferentially processed over cognitive information (Bar-Haim *et al.*, 2007). This improvement in learning and memory in elevated plus maze may result from the ability of the extract to preferentially enhance cognitive processing of information over anxiety that would disrupt cognitive performance.

In the Banes maze the extract at 1000 mg/kg and piracetam 400 mg/kg significantly ($p \leq 0.05$) decreased primary latency but non-significantly decreased total latency. The extract increased primary latency at 500 mg/kg. There was no change in total latency at 250 mg/kg. The extract significantly decreased primary and total errors at 250 mg/kg ($p \leq 0.05$) and 1000 mg/kg ($p \leq 0.05$ and $p \leq 0.01$ respectively). There was a non-significant increase in primary and total errors at 500 mg/kg. Piracetam non-significantly decreased total and primary errors. The extract significantly improved the time spent in target quadrant at 250 and 500 mg/kg ($p \leq 0.01$ and $p \leq 0.01$ respectively). There was a non-significant increase in time spent in target quadrant at 1000 mg/kg and with piracetam (Table 1). Piracetam and the extract at 1000 mg/kg improved spatial learning as indicated by decrease in escape latency. The extract at 250 and 1000 mg/kg and piracetam improved working memory by their ability to decrease escape errors. Similarly, the extract at all doses and piracetam improved reference memory as indicated by increasing time spent in the target quadrant. The Barnes maze is a test for the assessment of spatial learning and memory and relies on hippocampal-dependent spatial reference memory (Akirav *et al.*, 2001; Barnes, 1979; Brickman and Stern, 2009).

Table 1: Effect of methanol stem extract of *P. nigrescens* on escape latencies, escape errors, and time spent in target quadrant of mice in barnes maze.

Treatment groups	PL (S)	TL (S)	PE	TE	TSTQ (S)
1% G.A (10 ml/kg)	57.75±6.56	66.42±6.79	10.58±1.06	10.58±0.77	27.17±4.72
Piracetam (400 mg/kg)	37.54*±2.92	54.96±4.85	6.83±0.94	7.25±0.98	43.17±6.78
MSEPN (250 mg/kg)	61.21±8.51	69.01±9.01	5.00*±0.82	6.12*±0.99	53.17**±5.94
MSEPN (500 mg/kg)	58.54±9.65	91.17±11.33	12.67±2.01	14.29±2.13	62.00**±3.98
MSEPN (1000 mg/kg)	36.08*±2.92	54.04±3.99	4.54**±0.45	5.71*±0.36	34.17±3.58

Data presented as mean ± standard error of mean (n = 6); *$p < 0.05$; ** $p < 0.01$ as compared to 1 % G.A group; PL = primary latency; TL = total latency; TE: total error; PE: primary error; TSTQ: time spent in target quadrant; S: seconds; MSEPN: methanol stem extract of *P. nigrescens*

In the novel object recognition test, the methanol stem extract of *P. nigrescens* significantly ($p \leq 0.01$) increased discrimination at 500 and 1000 mg/kg. Piracetam significantly ($p \leq 0.05$) increased object discrimination index (Figure 2). The extract at all doses tested improved recognition memory as indicated by increase in discrimination index in the novel object recognition test. Substances capable of increasing discrimination index are known to improve recognition memory in NORT (Baxter, 2010). The novel object recognition test is used to assess recognition memory in rodents. The perirhinal, entorhinal and inferior cortices play an important role in object recognition memory (Hammond *et al.*, 2004; Aggleton *et al.*, 2010) and damages affecting these areas impair performance in recognition memory tasks (Albasser *et al.*, 2009). Drugs capable of improving recognition memory affect these brain regions.

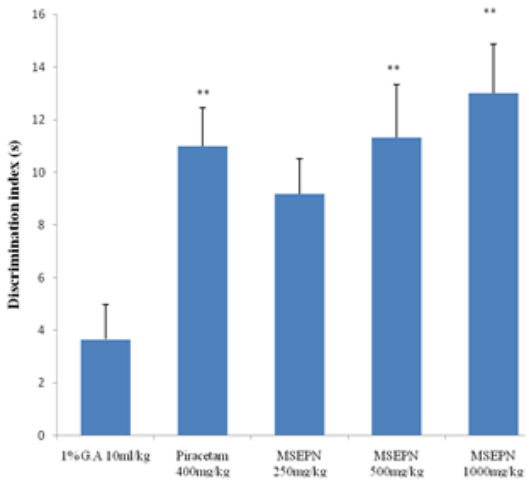

Figure 2: Effect of methanol stem extract of *P. nigrescens* on object discrimination index of mice in novel object recognition test. Data presented as mean ± standard error of mean; (n=6); *$p \leq 0.05$; **$p \leq 0.01$ as compared to 1% G.A; S: seconds; DI: discrimination index; G.A: gum acacia; MSEPN: methanol stem extract of *P. Nigrescens*.

In the open field, the extract at all doses did not significantly alter the number of square cross and rearing. Piracetam non-significantly increased the number of square cross and significantly ($p \leq 0.05$) increased number of rearing in the open field test (Table 2). In the open field test, the extract at all the doses did not alter locomotory activity of mice suggesting that, it is devoid of central nervous system stimulating or depressive effect. Locomotive effect of a substance affects cognitive parameters (Lowry *et al.*, 2005). In general, animals with impaired motor function will display decreased activity and vice versa (Nagaraju *et al.*, 2010). Decrease or increase in motor activity creates a false positive or negative result. The lack of stimulating and depressive effect of the extract indicates that the observed cognitive parameters in elevated plus maze, Barnes maze and novel object recognition tests are true cognitive enhancing ability of the methanol stem extract of *P. nigrescens*.

Table 2: Effect of methanol stem extract of *Parquetina nigrescens* on square cross and rearing of mice in an open field test.

Treatment groups	NSC	NR
1% G.A (10ml/kg)	87.50±4.97	32.17±2.30
Piracetam (400 mg/kg)	110.17±6.46	41.50*±2.14
MSEPN (250 mg/kg)	86.83±8.74	30.00±2.89
MSEPN (500 mg/kg)	90.00±3.83	31.00±2.56
MSEPN (1000 mg/kg)	92.17±8.00	35.17±2.20

Data presented as mean ± standard error of mean (n=6); *$p \leq 0.05$; **$p \leq 0.01$ as compared to 1% G.A; NSC: number of square cross; NR: number of rearing; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens.*

In the interoceptive model of elevated plus maze, the extract at all doses significantly ($p \leq 0.05$) decreased the transfer latencies increased by diazepam on day 1 and non-significantly on day 2. Piracetam significantly ($p \leq 0.01$, $p \leq 0.05$) decreased the transfer latencies on day 1 and 2 respectively increased by diazepam in an elevated plus maze (Fig.3). The ability of the methanol stem extract of *P. nigrescens* and piracetam to decrease the transfer latencies increased by diazepam implies reversal of diazepam induced cognitive deficit. The reversal of diazepam induced cognitive deficit suggests the cognitive enhancement may possibly be mediated through inhibition of the GABAergic system. Diazepam induces cognitive deficit through potentiation of GABAergic pathways. GABA antagonists are known to be strong memory activating agents. Administration of flumazenil a benzodiazepine antagonist or inverse agonist like methyl- and butyl-beta-carboline 3-carboxylate resulted in significant improvement of memory and learning in various tests (Kalueff and Nutt, 1996).

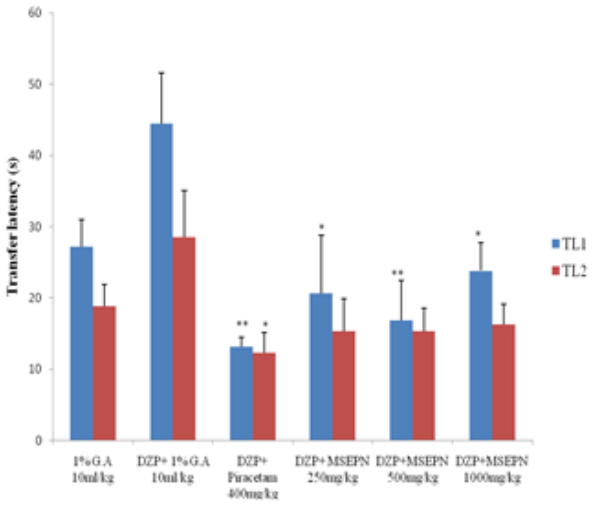

Figure 3: Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency of diazepam-induced amnesic mice in an elevated plus maze. Data presented as mean ± standard error of mean; (n=6); ** denotes $p \leq 0.01$; * denotes $p < 0.05$ as compared to DZP+1% G.A group; TL1: learning; TL2: memory; DZP: diazepam; S: seconds; G.A: gum acacia; MSEPN: methanol stem extract of *P. Nigrescens.*

The preliminary phytochemical screening of the methanol stems extract of *P. nigrescens* revealed the presence of carbohydrate, unsaturated sterol, tannins, saponins and phenolics which have been reported to have various pharmacological effects (George *et al.*, 2014; Rajeshwari *et al.* 2014). These phytochemicals constituents may be responsible for the observed learning and memory enhancing-like activity of *P. nigrescens.* The high oral median lethal dose greater than 5000 mg/kg is indicative of the safety of the methanol stem extract of *P. nigrescens* (Matsumura, 1985).

4. Conclusion

From the results of the study above, the methanol stem extract of *P. nigrescens* possesses learning and memory enhancing-like effect. This may provide the pharmacological credence to the ethnomedicinal use of the plant as memory enhancer.

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