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Pharmacological, phytochemical and *in-vivo* toxicological perspectives of a xero-halophyte medicinal plant: *Zaleya pentandra* (L.) Jeffrey

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ABSTRACT

This study endeavours to investigate the phytochemical composition, biological properties and *in vivo* toxicity of methanol and dichloromethane extracts of *Zaleya pentandra* (L.) Jeffrey. Total bioactive contents, antioxidant (phosphomolybdenum and metal chelating, DPPH, ABTS, FRAP and CUPRAC) and enzyme inhibition (cholinesterases, tyrosinase α-amylase, and α-glucosidase) potential were assessed utilizing *in vitro* bioassays. UHPLC-MS phytochemical profiling was carried out to identify the essential compounds. The methanol extract was found to contain highest phenolic (22.60 mg GAE/g) and flavonoid (31.49 mg QE/g) contents which correlate with its most significant radical scavenging, reducing potential and tyrosinase inhibition. The dichloromethane extract was most potent for phosphomolybdenum, ferrous chelation, α-amylase, α-glucosidase, and cholinesterase inhibition assays. UHPLC-MS analysis of methanol extract unveiled to identify 11 secondary metabolites belonging to five sub-groups, i.e., phenolic, alkaloid, carbohydrate, terpenoid, and fatty acid derivatives. Additionally, *in vivo* toxicity was conducted for 21 days and the methanol extract at different doses (150, 200, 250 and 300 mg/kg) was administered in experimental chicks divided into five groups each containing five individuals. Different physical, haematological and biochemical parameters along with the absolute and relative weight of visceral body organs were studied. Overall, no toxic effect was noted for the extract at tested doses.

1. Introduction

Natural products/or herbal medicines, comprising whole plants/herbs/spices or natural semi-synthetic drugs have been used for decades in the primary health care system in the management and/or treatment of common human ailments (Locatelli et al., 2018). Such folklore traditional practices usually originated from countries such as Africa, Latin America, and Asia amongst others. Nevertheless, such botanical remedies have now spread all through the global markets. At present, medical professionals, as well as consumers, have started to entrust and rely on phytomedicines, even replacing conventional clinically tested medications with such traditional and unconventional therapies (Thomford et al., 2018; Mocan et al., 2018a, 2018b; Diuzheva et al., 2018).

Moreover, the present research also tends to support the utilization of such bioresources for drug development programs as these phytochemicals commenced new horizons and potential to discover novel bioactive molecules with multiple pharmacological activities (Tatsumi

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et al., 2018; Yi et al., 2018). In traditional medicine of many countries, natural medicinal plants and herbs are mainly utilized as a significant alternative treatment approach for common ailments (Wu et al., 2018). The recent studied had reported that about 80% of the world's population relies on conventional therapies to treat their diseases, especially in underdeveloped countries and regions (Xiang et al., 2015). In the last few years, herbal medicines with natural therapies are gaining much popularity both in developed and developing countries, which may be due to herbal medicines, with thousands of years' usage, are comparatively safer than synthetic drugs for humans and/or due to the weak therapeutic effects of chemical drugs for some common health disorders (Ekor, 2014; Jordan et al., 2010). Although, maximum natural products which are utilized in traditional medicine have insufficient scientific validation and pharmacological studies specifically regarding their efficacy and safety (Xiang et al., 2015) as these herbal drugs are mostly used in human beings without testing their potential toxicity, which practically does not warrant their safety (Traesel et al., 2016). The global increase in consumption of herbal drugs has resulted in increasing concerns regarding their safety. Therefore, significant efforts have been made in order to test the toxicological parameters of many natural products using several standard assays.

Zaleya pentandra (L.) Jeffrey. (Family: Aizoaceae) is a xero-halophyte perennial plant growing on coastal and near-coastal sandy salt flats of Asian and African countries including Pakistan, Iran, India, and Africa (Ehsen et al., 2017). It has folklore uses to treat snakebites, cough and stomach disorders. The plant has also described being efficacious against gonorrhoea and respiratory tract infections due to the presence of some steroids (Afzal et al., 2013; Bhatti et al., 2001; Khan and Qaiser, 2006; Qasim et al., 2010). However, there is still a paucity of research data on the phytochemical composition, biological activities and the toxicological evaluation of this plant. This research work was thus designed in order to investigate for the first time into the biological properties, chemical profile, and safety or toxicity potential of *Z. pentandra* methanol and dichloromethane extracts. The biological potential was determined using multiple *in-vitro* antioxidant assays including DPPH, ABTS (radical scavenging), FRAP, CUPRAC (reducing power), phosphomolybdenum and metal chelation.

Similarly, inhibition capabilities of both the extracts against essential enzymes involved in neurodegenerative diseases (AChE, BChE), diabetes (α-amylase and α-glucosidase and skin problems (tyrosinase) were also assessed. Besides, *in-vivo* toxicity study was conducted on chicks in order to report the safety parameters for this plant. It is assumed that the findings of current research will present a valuable contribution toward establishing baseline data and highlighting the potential application for its therapeutic effects of *Z. pentandra.* This study has provided insights into the potential therapeutic uses of *Z. pentandra*, which could open new avenues for novel pharmaceuticals.

2. Material and methods

2.1. Plant material

The plant *Z. pentandra* was collected in April from Desert of Cholistan, Pakistan. The plant was identified as *Z. pentandra* by Mr. Hafiz Waris, Taxonomist, The Islamia University of Bahawalpur, Cholistan Institute of Desert Studies (CIDS). A voucher specimen of the plant material (ZP-WP-01-16-173) was also deposited in the herbarium of Faculty of Pharmacy and Alternative Medicines, The Islamia University of Bahawalpur for future reference. The plant material was firstly shade-dried and then subjected to extraction (maceration) successively using dichloromethane and methanol solvents at room temperature (72 h). Pooled extracts were dried using Rotary evaporator-R20 at 35 °C. These extracts were abbreviated as; ZP-M (*Z. pentandra* methanol extract), ZP-D (*Z. pentandra* dichloromethane extract).

2.2. Total bioactive contents and UHPLC-MS analysis

Total phenolics were determined by employing a standard Folin-Ciocalteu method (Zengin et al., 2018b) using gallic acid as standard and the results were expressed as mg GAE/g (gallic acid equivalents). While, total flavonoid content assay was done by aluminium chloride spectrophotometric method (Chew et al., 2009). Quercetin was used as a standard, and the results were expressed as mg QE/g (quercetin equivalent). Both these tests, based on spectrophotometric analysis, could allow obtaining preliminary information on the total bioactive compounds (phenolics and flavonoids). These procedures, also applied in other papers (Uysal et al., 2017; Saleem et al., 2019) were carried out by using well-known procedure as follow. Two fifty microlitres of the sample solution (1 mg/mL) was mixed with the diluted (1:9, v/v) Folin–Ciocalteu reagent (1 mL). 0.75 mL of a Na_2CO_3 solution (1%) was added after 3 min and then the sample absorbance was read at 760 nm after 2 h of incubation at room temperature. For the total flavonoids content, 1 mL of the sample solution (1 mg/mL) was mixed with an equal volume of aluminium chloride (2%) solution in methanol and the absorbance was read at 415 nm after 10 min of incubation at room temperature. Quercetin was used as a reference standard and the total flavonoid content was expressed as milligrams of quercetin equivalents (mg QE/g extract).

Secondary metabolites profiling was done by RP-UHPLC-MS (reversed phase ultra-high performance liquid chromatography-mass spectrometry) analysis. Agilent 1290 Infinity UHPLC system which is coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer interfaced with a dual ESI source was utilized. The column used was Agilent Zorbax Eclipse XDB-C18 (2.1 × 150 mm, 3.5 μm). The temperature of 4 °C and 25 °C was maintained for auto-sampler and column respectively. The mobile phase (A) used was 0.1% formic acid solution in water, and acetonitrile and 0.1% formic acid solution was the mobile phase (B). The flow rate of mobile phase was kept at 0.5 mL/min. Plant extract solution (1.0 μL in HPLC grade methanol solvent) was injected for the time of 25 min, and 5 min were used for post-run time. Nitrogen gas with a flow rate of 25 and 600 L/hour was used as a source of nebulizing and drying gas respectively and the temperature was maintained at 350 °C. The analysis was performed with a capillary voltage of 3500 V while the fragmentation voltage was optimized to 125 V.

2.3. Antioxidant assays

The free radical scavenging (DPPH, ABTS), reducing power (FRAP, CUPRAC) phosphomolybdenum (total antioxidant capacity), and metal chelating (ferrous ion chelation) were evaluated following the previous methods as described by Grochowski et al. (2017) (Grochowski et al., 2017).

2.3.1. Free radical scavenging activity (DPPH)

One milli litre of the sample solution was added to the DPPH solution (0.267 mM 4 mL, 0.004% methanol solution), and after 30 min of incubation, the absorbance was recorded at 517 nm. Milligrams of trolox equivalents per gram of dry extract (TEs/g extract) were the measure unit.

2.3.2. ABTS radical cation scavenging activity

$ABTS^+$ radical cation was obtained following the reaction between 7 mM ABTS solution and 2.45 mM potassium persulfate. One milli litre of the test solution was mixed with 2 mL of ABTS solution and after 30 min the absorbance was recorded at 734 nm. The results were expressed as milligrams of trolox equivalents per gram of dry extract (TEs/g extract).

2.3.3. Phosphomolybdenum method

Three hundred micro litre of the sample solutions were mixed with

3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), and after 90 min the absorbance was recorded at 695 nm. Millimoles of trolox equivalents per gram of dry extract (TEs/g extract) were the measurement unit.

2.3.4. Cupric ion reducing (CUPRAC) method

Five hundred micro litre of the sample solutions were mixed with [CuCl2 (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM), NH_4Ac buffer (1 mL, 1 M, pH 7.0)] and after 30 min the absorbance was recorded at 450 nm. Milligrams of trolox equivalents per gram of dry extract (TEs/g extract) were the measurement unit.

2.3.5. Ferric reducing antioxidant power (FRAP) method

One hundred micro litre of the sample solution was added to reagent (2 mL) in acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a final ratio of 10:1:1 (v/v/v) and the absorbance was recorded at 593 nm 30 min. Milligrams of trolox equivalents per gram of dry extract (TEs/g extract) were the measurement unit.

2.3.6. Metal chelating activity on ferrous ions

Two hundred micro litre of the sample solution were added to 0.05 mL of a solution of $FeCl_2$ (2 mM), then the reaction was initiated using 0.2 mL of ferrozine (5 mM). After 10 min the absorbance was recorded at 562 nm and the results expressed as milligrams of EDTA equivalents per gram of dry extract (EDTAEs/g extract).

2.4. Enzyme inhibition assays

The enzyme inhibition activities of the extracts against acetylcholinesterase, butyrylcholinesterase, α-amylase, α-glucosidase, and tyrosinase were assessed utilizing previously described established *in-vitro* methods (Grochowski et al., 2017). The AChE and BChE inhibition activity were expressed as equivalents of galanthamine as standard (mg GALAE/g extract), while acarbose equivalent (mmol ACAE/g extract) for α-glucosidase and α-amylase and kojic acid equivalent (mg KAE/g extract) for tyrosinase were used.

2.4.1. Cholinesterase

The reaction mixture composed by the sample solution (50 μL), DTNB (3 mM 125 μL) and enzyme solution (0.265 u/mL AChE or 0.026 u/mL BChE) solution (25 μL) in Tris-HCl buffer (pH 8.0) was added to the substrates [acetylthiocholine iodide (15 mM ATCI) or butyrylthiocholine chloride (1.5 mM BTCl, 25 μL)]. After 15 min of incubation, the absorbance was recorded at 405 nm and the results expressed as milligrams of galantamine equivalents per gram of dry extract (GALAEs/g extract).

2.4.2. α-Amylase

The reaction mixture composed by 25 μL of the sample solution and 50 μL of the α-amylase solution (10 u/mL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) was added to 50 μL of the starch solution (0.05%) and the reaction was stopped with the addition of 25 μL of HCl (1 M). Then 100 μL of the iodine-potassium iodide solution was added. After 10 min of incubation, the absorbance was recorded at 630 nm and the results expressed as millimoles of acarbose equivalents per gram of dry extract (ACAEs/g extract).

2.4.3. α-Glucosidase

Fifty micro litre of the sample solution and glutathione (0.5 mg/mL, 50 μL), α-glucosidase solution (0.2 u/mL 50 μL) in phosphate buffer (pH 6.8) and PNPG (10 mM, 50 μL) was stopped after 15 min with 50 μL of a sodium carbonate solution (0.2 M) and the absorbance was recorded at 400 nm. The results were expressed as millimoles of acarbose equivalents per gram of dry extract (ACAEs/g extract).

Table 1
Grouping and treatments for toxicity studies.

Groups	Treatments
Group 1	Control (10 mL/kg Normal Saline).
Group 2	ZP extract 150 mg/kg BW
Group 3	ZP extract 200 mg/kg BW
Group 4	ZP extract 250 mg/kg BW
Group 5	ZP extract 300 mg/kg BW

2.4.4. Tyrosinase

Twenty five micro litre of the sample solution were added to a 40 μL of tyrosinase solution (200 u/mL) and phosphate buffer (40 mM, 100 μL, pH 6.8) in a 96-well microplate and incubated for 15 min at 25 °C. Then the reaction was initiated by adding l-DOPA (10 mM, 40 μL). After 10 min of incubation at room temperature, the absorbance was recorded at 492 nm and the results expressed as milligrams of kojic acid equivalents per gram of dry extract (KAE/g extract).

2.5. In vivo toxicological studies

2.5.1. Animals

This study was executed on a-day old broiler chicks (n = 50). The birds were kept under similar management and housing conditions. All chicks were vaccinated according to schedule for Newcastle (ND), Infectious Bursal disease (IBD) and Hydropericardium (HPS). Basal diet, i.e., chick starter mash, 22% total protein and cleaning drinking water was offered *ad libitum* to these birds. After 7 days of acclimatization, the experimental chicks were divided randomly into 5 equal groups. The treatment was started and continued to day 21 (end of experiment) on a daily basis as provided in Table 1.

2.5.2. Toxicity parameters studied

The toxicity studies were carried out as reported earlier with slight modifications (Ghaffar et al., 2017; Shahzad et al., 2012). Clinical signs in all the groups were observed and recorded for any abnormality due to *Z. pentandra* extract toxicity. Behavioural alterations in all the groups were observed. Different parameters of behavioural alterations included, the alertness of the birds, the response on stimulus, foaminess in faces and shininess of feathers were also observed. Body weight of all birds was recorded on day 21. Birds of each group were slaughtered on day 21 for the following studies; (1) Gross examination of all body tissues, and (2) recording of absolute and relative organ weight of liver, lungs, kidney, heart, intestine, thymus, trachea, and bursa.

2.5.3. Haematological and biochemical analysis

The automatic haematological analyser was used to perform Haematological analysis was. The studied parameters comprise erythrocytes, leucocyte, lymphocyte, haemoglobin, MCV, MCHC and haematocrit (HCT) count. Similarly, in the case of biochemical analysis, the blood was centrifuged at 3000 rpm for 10 min. Serum was separated and stored at 20 °C until the determination of serum creatinine, urea, alkaline phosphate, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

2.5.4. The absolute and relative weight of body organs

The killing of chicks from each group was carried out by neck dislocation on day 21. After killing of an individual bird, visceral organs (liver, kidney, lungs, intestine, heart, trachea, thymus and bursa) were weighed to measure the absolute weight of each organ. The relative weight of those organs was calculated as the percent of body weight in individual birds.

2.6. Statistical analysis

All the experiments were executed three times, and analysis was

Table 2
Total bioactive contents and antioxidant properties of *Z. pentandra* extracts.

Bioactive contents and antioxidant assays		ZP-M	ZP-D
Extraction yield (%)		17%	15%
Total bioactive contents	Total phenolic content (mg GAE/g)	22.60 ± 0.14	18.45 ± 1.05
	Total flavonoid content (mg QE/g)	31.49 ± 0.29	12.09 ± 0.23
Radical Scavenging activity	DPPH (mg TE/g extract	25.60 ± 1.01	3.56 ± 0.96
	ABTS (mg TE/g extract)	45.14 ± 1.89	10.29 ± 0.67
Reducing power	FRAP (mg TE/g extract))	50.01 ± 0.99	33.22 ± 1.37
	CUPRAC (mg TE/g extract)	95.93 ± 3.13	86.43 ± 2.31
Total antioxidant capacity	Phosphomolybdenum (mg TE/g extract)	1.08 ± 0.05	1.49 ± 0.02
Ferrous chelating	Metal Chelating (mg EDTAE/g)	15.99 ± 1.11	54.63 ± 1.07

Data from three repetitions, with mean ± standard deviation; GAE: gallic acid equivalent; QE: quercetin equivalent; TE: trolox equivalent; EDTAE: EDTA equivalent.

done in triplicates. The obtained results were presented as mean value and standard deviation (mean ± SD). One-way analysis of variance (ANOVA) was used to calculate the differences, followed by Tukey's significant difference post hoc test ($p < 0.05$). SPSS v22.0 software was used to carry out all experimental analysis.

3. Results and discussion

3.1. Phytochemical composition and antioxidant activities

Total phenolic and flavonoid contents were measured in terms of standard Gallic acid (GAE) and Quercetin (QE) equivalent, respectively. The results are depicted in Table 2. The methanol extract was found to contain higher phenolic (22.60 ± 0.14 mg GAE/g) and flavonoid (31.49 ± 0.29 mg QE/g) contents.

At present, the discovery of novel and efficacious antioxidants of natural origin has been given prime importance to prevent oxidative stress and related diseases (Zengin et al., 2018b). The antioxidant capacity of methanol and DCM extracts of *Z. pentandra* in terms of its free radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelation activity are shown in Table 2. The methanol extract containing the highest total bioactive contents showed the highest radical scavenging (DPPH: 25.60 ± 1.01 mgTE/g extract; ABTS: 45.14 ± 1.89 mgTE/g extract) and reducing power (FRAP: 50.01 ± 0.99 mgTE/g extract CUPRAC: 95.93 ± 3.13 mgTE/g extract) capacities. A similar trend was described by some other studies, which described a strong correlation among radical scavenging ability and total phenolic contents of different plant extracts and also reported that phenolic compounds are responsible for playing a major contribution as antioxidants (Khan et al., 2016; Žugić et al., 2014).

The total antioxidant capacity of *Z. pentandra* extracts was further estimated by the phosphomolybdenum assay, which is regarded as a meaningful and simple method and is mostly used to measure the antioxidant power of isolated pure compounds and plant extracts (Llorent-Martínez et al., 2017). However, interestingly, as mentioned in Table 2, the DCM extract exhibited the highest phosphomolybdenum while the methanol extract was least effective. As the phosphomolybdenum antioxidant assay measures the antioxidant capacity of both phenolic and non-phenolic compounds, so the observed results regarding this assay can be associated with the presence of non-phenolic antioxidant compounds in dichloromethane extracts. Therefore, in this case, non-phenolic antioxidants such as vitamin C or carotenoids might be responsible for the observed total antioxidant capacity, as also previously reported by some other studies (Albayrak et al., 2010; Llorent-Martínez et al., 2017; Mocan et al., 2015). The similar trend was also observed in the case of the metal chelating assay, and the dichloromethane extract (54.63 ± 1.07 mg EDTAE/g extract) was found to be more efficient in this assay than methanol. However, ZP-M exhibited higher radical scavenger potential, reducing power capacities and total bioactive contents. From this study, it is assumed that the metal chelation ability of plant extracts are not only associated with the phenolic and flavonoids, but also on the existence of some other secondary metabolites like polysaccharides, ascorbic acid, tocopherols, and peptides. Our results can be further argued by the studies which reported that the metal chelation capacity contributes only a minor role in the overall antioxidant potential of phenolic compounds (Rice-Evans et al., 1996). Moreover, previous researchers have also reported a weak correlation between phenolic contents and metal chelation (Chavan et al., 2013; Llorent-Martínez et al., 2017).

Liquid chromatography-mass spectrometry analysis of the *Z. pentandra* methanol extract was performed to have an insight into the possible secondary metabolite components. The UHPLC-MS analysis of *Z. pentandra* methanol extract has revealed the presence of 11 phytocompounds (Table 3) and its MS total ion chromatogram is shown in Fig. 1. The main compounds were phenol (caffeic acid 3-glucoside, gingerol), an alkaloid (3-O-acetylhamayne and salannin), triterpenoid (ganoderic acid H) and carbohydrate (vanilloloside) derivatives. Moreover, three fatty acids 9Z, 12Z, 15E-octadecatrienoic acid, 2-Hydroxyhexadecanoic acid, 6E, 9E-octadecadienoic acid and one Cyanogenic compound (sarmentosin epoxide) were also identified. The presence of carbohydrates, alkaloids, phenolics, and terpenoids in the

Table 3
UHPLC-MS analysis of *Z. pentandra* methanol extract.

S. No	RT (min)	Base Peak (m/z)	Proposed compounds	Compound class	Mol. Formula	Mass
1	0.925	290.09	Sarmentosin epoxide	Cyanogenic compound	C11 H17 N O8	291.09
2	7.644	315.11	Vanilloloside	Carbohydrate	C14 H20 O8	316.11
3	8.063	341.09	Caffeic acid 3-glucoside	Hydroxycinnamic acid	C15 H18 O9	342.09
4	9.24	328.12	3-O-Acetylhamayne	Alkaloid	C18 H19 N O5	329.12
5	11.998	623.24	Grossamide	Lignan amide	C36 H36 N2 O8	624.24
6	13.293	293.18	Gingerol	Phenol	C17 H26 O4	294.18
7	15.702	595.29	Salannin	Alkaloid	C34 H44 O9	596.29
8	16.763	571.29	Ganoderic acid H	Triterpenoid	C32 H44 O9	572.29
9	18.434	277.22	9Z,12Z,15E-octadecatrienoic acid	Fatty acid	C18 H30 O2	278.22
10	18.5	271.23	2-Hydroxyhexadecanoic acid	Fatty acid	C16 H32 O3	272.23
11	19.358	279.24	6E,9E-octadecadienoic acid	Fatty acid	C18 H32 O2	280.24

RT: retention time.

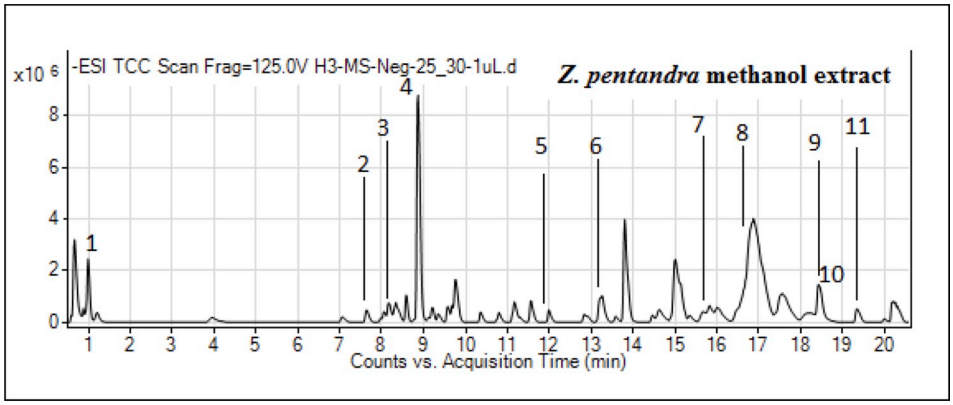

Fig. 1. Total ion chromatogram (TIC) of *Z. pentandra* methanol extract.

extracts is in line with other Zaleya species (Chaudhari, 1996; Khare, 2008; Meenakshi et al., 2010). This is the first report on the preliminary UHPLC-MS secondary metabolite composition of *Z. pentandra* methanol extract.

3.2. Enzyme inhibition studies

One of the most promising strategies to combat with the global health-related problems is to inhibit the key enzymes involved in the specific disorders. For example, α-amylase and α-glucosidase inhibition for treating diabetes mellitus, cholinesterases inhibition for neurodegenerative diseases and tyrosinase inhibition for skin problems, amongst others. The major problem with the synthetic drugs presently used for treating these diseases is their associated side effects (Zengin et al., 2016, 2017). Surprisingly, herbs, plants and their plethora of secondary metabolites have proved to be potential inhibitors of these specific enzymes. In this sense, the inhibition potential of *Z. pentandra* extracts against AChE, BChE, tyrosinase, α-amylase, and α-glucosidase were tested, and the results are presented in Table 4. In the case of cholinesterases, both the extracts showed significant inhibition against AChE and BChE. The DCM extract showed the higher AChE (4.47 ± 0.19 GALAE/g extract) and BChE inhibition (4.69 ± 0.84 mg GALAE/g extract). This observed higher inhibition by DCM extracts might be correlated to the existence of non-phenolic compounds like alkaloids which have been previously reported for cholinesterase activities (Parveen et al., 2001; Yan et al., 2011).

Similarly, for α-amylase and α-glucosidase, the DCM extracts (1.53 ± 0.09 and 1.88 ± 0.01 mmol ACAE/g extract, respectively) showed the highest inhibition compared to methanol (0.51 ± 0.02 and 0.61 ± 0.01 mmol ACAE/g extract, respectively) extract (Table 4). Previously, Meenakshi et al. (2010)has reported the ethanol extract (200 mg/kg body weight) of *Z. decandra* roots to produce hypoglycemic effect when given orally to alloxan induced diabetic rat for 15 days and they had also reported that the studied extract induces an increase in the release of insulin and restoring metabolic properties hence causing hypoglycemic effects (Meenakshi et al., 2010).

Table 4
Enzyme inhibition activities of *Z. pentandra* extracts.

Enzymes	ZP-M	ZP-D
AChE inhibition (mg GALAE/g extract)	4.05 ± 0.18	4.47 ± 0.19
BChE inhibition (mg GALAE/g extract)	4.16 ± 0.29	4.69 ± 0.84
α-amylase (mmol ACAE/g extract)	0.51 ± 0.02	1.53 ± 0.09
α-glucosidase (mmol ACAE/g extract)	0.61 ± 0.01	1.88 ± 0.01
Tyrosinase (mg KAE/g extract)	126.17 ± 1.78	111.97 ± 0.67

GALAE: galantamine equivalent; KAE: kojic acid equivalent; ACAE: acarbose equivalent; All values expressed are means ± S.D. of three parallel measurements.

Besides, both the extracts exhibited high tyrosinase inhibition (mean inhibition of 126.17 ± 1.78 mg KAE/g extract) was found in methanol extract followed by DCM extract (with a value of 111.97 ± 0.67 mg KAE/g (Table 4). This highest tyrosinase inhibition by phenolic-rich methanol extract can be correlated to previous studies which described a strong correlation of TBC and tyrosinase inhibition (Zengin et al., 2018a). Polyphenolic compounds are also reported to reversibly bind to tyrosinase enzyme and thus resulting in a reduction of its catalytic capacity (Chang, 2009). From the present experiments, the *Z. pentandra* extracts presented significant antioxidant and enzyme inhibition potential.

3.3. Toxicity studies of Z. pentandra

The *in-vivo* toxicological impact of *Z. pentandra* methanol extract was evaluated up to dose 300 mg/kg b. w. for the extended period by studying different haematological, biochemical and physical parameters. Chicks divided into five groups received different doses for 21 days by the oral route of administration to evaluate safety protocols. Clinical signs and behavioural changes were notified in all experimental chicks divided into different groups (Table 5). No negative clinical sign and behaviour were observed in any individual among all groups at any dose until the completion of the study. No toxicity signs or deaths were recorded during the 21 consecutive days of treatment. There were no changes in their general behaviour or other physiological conditions such as pain sensibility, locomotion and body temperature. Moreover, the animals did not present considerable variation in the body weights among control and treated groups. Similarly, in the case of water and food consumption, no toxic effect was seen.

Similarly, as the haematopoietic system is one of the most sensitive targets for toxic chemicals and an essential index of physiological and pathological status in humans and animals (Nghonjuyi et al., 2016), the effects of oral administration of *Z. pentandra* methanol extract (150, 200, 250 and 300 mg/kg) on the blood parameters were studied at days 7, 14 and 21 and the results are summarized in Table 6. All studied

Table 5
Effect of oral administration of methanol and extract of *Z. pentandra on* clinical signs in chicks.

Groups	Treatment	Clinical signs			
		Depression	Response	Feeding	Skin lesion
1	Control	–	+ +	+	–
2	150 mg/kg BW	–	+ +	+	–
3	200 mg/kg BW	–	+ +	+	–
4	250 mg/kg BW	–	+ +	+	–
5	300 mg/kg BW	–	+ +	+	–

No sign (−), good (+), best (+ +).

Table 6
Effect of oral administration of methanol extract of *Z. pentandra* on haematological parameters of chicks.

Parameters/Day	Groups (ZP)				
	Control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
Erythrocyte counts					
Day 7	2.48 ± 0.11	2.63 ± 0.087	2.51 ± 0.01	2.69 ± 0.09	2.54 ± 0.14
Day 14	2.63 ± 0.14	2.46 ± 0.079	2.47 ± 0.06	2.65 ± 0.08	2.58 ± 0.06
Day 21	2.494 ± 0.07	2.54 ± 0.07	2.41 ± 0.05	2.48 ± 0.05	2.50 ± 0.05
Leucocyte Count (103/μL)					
Day 7	197.38 ± 3.62	193.96 ± 2.45	192.86 ± 1.62	195.04 ± 0.94	208.86 ± 3.47
Day 14	201.92 ± 10.63	196.16 ± 3.73	229.14 ± 15.33	216.28 ± 9.93	214.78 ± 15.20
Day 21	214.1 ± 6.93	221.3 ± 3.82	228.18 ± 15.01	213.18 ± 9.23	224.8 ± 15.84
Lymphocyte Count (103/μL)					
Day 7	181.36 ± 13.82	168.98 ± 2.60	204.98 ± 17.46	218.58 ± 7.37	180.1 ± 11.34
Day 14	218.1 ± 2.78	170.42 ± 1.67	186 ± 11.86	223.08 ± 1.27	219.62 ± 2.67
Day 21	215.22 ± 12.41	209.86 ± 3.79	205.4 ± 18.12	202.62 ± 20.64	200.86 ± 21.9
Haemoglobin (gm/dL)					
Day 7	9.36 ± 0.29	10.5 ± 0.28	10.12 ± 0.28	9.7 ± 0.26	10.22 ± 0.34
Day 14	10.38 ± 0.52	10.34 ± 0.37	9.98 ± 0.17	10.8 ± 0.53	10.16 ± 0.21
Day 21	10.34 ± 0.41	10.54 ± 0.34	10.84 ± 0.29	11.18 ± 0.40	10.96 ± 0.33
MCV (FL)					
Day 7	130.92 ± 3.23	123.96 ± 1.33	133.4 ± 4.39	135.92 ± 2.72	134.1 ± 3.40
Day 14	134.1 ± 1.37	116.84 ± 0.77	136.04 ± 1.65	133.26 ± 1.25	135.48 ± 1.94
Day 21	136.36	137.38	133.96	137.54	139.66
MCHC (Pg)					
Day 7	32.66 ± 1.96	31.26 ± 0.14	31.64 ± 1.07	38.04 ± 1.77	36.02 ± 3.19
Day 14	34.34 ± 2.65	31.52 ± 0.40	30.08 ± 0.74	39.22 ± 2.11	36.5 ± 2.59
Day 21	32.64 ± 1.79	31.78 ± 0.18	31.24 ± 0.76	36.68 ± 1.29	35.1 ± 2.22
HCT (%)					
Day 7	31.8 ± 1.54	31.66 ± 0.65	34.4 ± 0.45	35.24 ± 0.76	33.56 ± 1.02
Day 14	33.44 ± 1.06	30.74 ± 0.87	32.7 ± 0.40	36.72 ± 0.99	35.84 ± 0.99
Day 21	33.2 ± 1.35	32.12 ± 0.68	33.66 ± 0.73	37.64 ± 1.03	33.36 ± 1.18

All values expressed are means ± S.D. of three parallel measurements. MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; HCT: haematocrit.

haematological parameters (erythrocytes, leucocytes, lymphocytes, haemoglobin, MCV and MCHC and HCT) remained within reasonable limits during the treatment period at all the tested doses and no significant differences between control and treated groups were noted. An analysis of blood parameters is apposite to risk evaluation as the changes in the haematological system have a higher predictive value for human toxicity when the data are extrapolated from animal studies (Olson et al., 2000). The results indicate that the methanol extract of *Z. pentandra* has no effects on the circulating blood cells as well as their production. However, there was the only a slight increase in leucocytes count was observed in all groups treated with *Z. pentandra* methanol extract (Table 6). As the increase in white blood cells directly indicates the strengthening of the organism's immune defence (Gefu et al., 2000), thus elevation in total leucocyte count suggests the *Z. pentandra* extract to contain biologically active principles that have the ability to boost the immune system through an increase in the number of defensive white blood cells. Overall, no significant difference was observed in experimental and control chicks, indicating the non-toxic nature of *Z. pentandra* plant even at higher doses.

Additionally, the biochemical profiles of the chicks treated with the methanol extract (150, 200, 250 and 300 mg/kg) and control were studied in all groups at day 21, and the results are presented in Table 7. Repeated oral administration of the *Z. pentandra* did not cause significant changes in serum creatinine, urea and the liver marker enzymes (ALP, ALT, and AST). No injury to anybody organs like kidney or liver was observed by administering even higher doses of the extract as compared with control. Moreover, the effect of orally administered *Z. pentandra* extract was also observed on the absolute and relative weight of visceral body organs (liver, kidney, lungs, thymus, intestine, heart, spleen, trachea, and bursa) of the chicks from all groups on day 21, as presented in Table 8. The result indicated no significant difference when compared with the control which showed that there was no toxic effect on absolute and relative weights of body organs. To conclude, the *Z. pentandra* methanol extract could be claimed to be non-toxic because no mortality was reported and no significant changes in haematological, biochemical and body weights of experimental chicks were observed throughout the 21 days of the toxicity evaluation study.

4. Conclusion

This is the first comprehensive study on the chemical composition, biological activities and *in-vivo* toxicity evaluation of *Z. pentandra*

Table 7
Effect of oral administration of methanol extract of *Z. pentandra* on biochemical parameters of chicks.

Biochemical parameters	Control	Plant extract			
		150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
Alkaline Phosphatase	1667 ± 3.7	1647 ± 1.9	1661 ± 4.5	1366 ± 1.16	1671 ± 2.3
Aspartate Aminotransferase	7.82 ± 1.2	8.75 ± 0.75	7.75 ± 0.75	9.25 ± 1.25	8.23 ± 0.23
Alanine Aminotransferase	162 ± 2.4	155 ± 1.5	168 ± 1.3	165 ± 2.7	151 ± 1.9
Urea level	19.23 ± 0.5	21.02 ± 2.3	15.9 ± 5.1	18.71 ± 4.75	23.75 ± 1.77
Creatinine	0.423 ± 0.01	0.38 ± 0.8	0.425 ± 0.12	0.3225 ± 0.03	0.312 ± 0.21

All values expressed are means ± S.D. of three parallel measurements.

Table 8
Effect of oral administration of methanol extract of *Z. pentandra* on body organs of chicks.

Parameters/Day	Groups (ZP)				
	Control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
Liver weight					
Absolute	40.02 ± 2.32	36.76 ± 0.68	49.05 ± 3.7	48.25 ± 4.79	47.5 ± 2.62
Relative	1.6978 ± 0.11	1.7000 ± 0.039	1.9751 ± 0.21	1.9726 ± 0.19	2.0272 ± 0.26
Kidney weight					
Absolute	4 ± 0.41	6.2 ± 1.93	7.1 ± 1.24	9.8 ± 2.57	6.93 ± 2.95
Relative	0.1694 ± 0.01	0.2937 ± 0.103	0.2888 ± 0.06	0.4002 ± 0.10	0.3116 ± 0.15
Lungs weight					
Absolute	10.57 ± 0.68	4.1 ± 0.40	10.54 ± 0.66	9.83 ± 1.17	10.71 ± 0.54
Relative	0.168 ± 0.015	0.1914 ± 0.027	0.1781 ± 0.01	0.377 ± 0.11	0.27 ± 0.09
Thymus weight					
Absolute	4.2 ± 0.45	4.53 ± 0.145	5.06 ± 0.17	4.93 ± 0.6	4.133 ± 0.46
Relative	0.18 ± 0.02	0.23 ± 0.01	0.14 ± 0.03	0.29 ± 0.03	0.22 ± 0.03
Intestine weight					
Absolute	151.22 ± 10.12	120.84 ± 17.22	159.7 ± 3.45	122.03 ± 5.68	144.2 ± 9.73
Relative	6.414 ± 0.47	5.542 ± 0.59	6.407 ± 0.33	4.999 ± 0.32	6.127 ± 0.72
Heart weight					
Absolute	10.16 ± 1.21	8.633 ± 0.27	11.3 ± 1.75	10.92 ± 1.103	9.753 ± 1.17
Relative	0.4299 ± 0.047	0.398 ± 0.004	0.4557 ± 0.07	0.4481 ± 0.052	0.4200 ± 0.08
Spleen weight					
Absolute	1.8666 ± 0.28	1.966 ± 0.16	2.366 ± 0.38	2.0433 ± 0.105	2.1226 ± 0.22
Relative	0.0790 ± 0.01	0.0916 ± 0.01	0.094 ± 0.015	0.0836 ± 0.005	0.08874 ± 0.04
Trachea weight					
Absolute	2.523 ± 0.31	2.6 ± 0.33	1.9 ± 0.39	3.46 ± 0.26	2.9 ± 0.44
Relative	0.1067 ± 0.006	0.1186 ± 0.01	0.076 ± 0.001	0.1421 ± 0.021	0.124 ± 0.02
Bursa weight					
Absolute	1.14 ± 0.14	0.983 ± 0.26	1.733 ± 0.18	1.03 ± 0.16	1.69 ± 0.25
Relative	0.048 ± 0.006	0.0465 ± 0.01	0.0699 ± 0.005	0.0420 ± 0.006	0.070 ± 0.006

All values expressed are means ± S.D. of three parallel measurements.

extracts. We found that the methanol extract possessed high total bioactive contents which we suggest can be correlated with its high free radical scavenging, reducing power and tyrosinase inhibition potentials while the dichloromethane extract shows valuable antioxidant, and metal chelation activities, coupled to an interesting activity on key-enzyme nowadays involved in diabetic disease. Additionally, UHPLC-MS profiling revealed the presence of 3-O-acetylhamayne, grossamide, salannin, and ganoderic acid as major phytochemicals present. The oral dose of methanol extract did not cause either mortality or adverse effects in the general behaviour in acute toxicity study and did not produce significant changes in almost all biochemical and haematological parameters. Consequently, *Z. pentandra* could be regarded as safer and a prime source for bioactive natural antioxidant and enzyme inhibitor that can be further explored to design new phytopharmaceuticals. Nevertheless, future studies in terms the mechanisms associated with observed biological activities, bioavailability and involved metabolic pathways are recommended.

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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