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Table of Contents: Volume 10 Number 5 3 February, 2016

ARTICLE

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Full Length Research Paper

Schistosomicidal and molluscicidal activities of two *Junipers* species cultivated in Egypt and the chemical composition of their essential oils

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In the present study, *in vitro* bioassay screening of total methanolic extracts of two *Juniperus* species (*Juniperus horizontalis* Moench. and *Juniperus communis* L.) cultivated in Egypt for schistosomicidal and molluscicidal activities was carried out. *Schistosoma mansoni* Sambon worms and *Biomphalaria alexandrina* (Ehrenberg) snails were used. The screening results showed that both tested extracts had almost similar schistosomicidal activity (LC50 ≈ 91 µg/ml, in 3 days) while *J. communis* extract possess more potent molluscicidal activity than *J. horizontalis* (LC_{50} = 22.9 and 38.9 ppm, after one day, respectively). Analysis of the chemical composition of the essential oils obtained by hydro-distillation of the aerial parts of the two *Junipers* species was done by GC/MS. Sixty seven (94.32%) components were identified in *J. communis* oil with homogeraniol (36.95%) being the major constituent, while sixty (95.39%) components were identified in *J. horizontalis* oil with the main component, bronyl acetate representing 41.17%.

Key words: Essential oils, *Juniperus*, molluscicidal, schistosomicidal.

INTRODUCTION

The Cupressaceae or Cypress family is a conifer family of cosmopolitan distribution. The family includes about 70 genera (17 monotypic) with about 130-142 species. One of the important genus is *Juniperus* which has been well known as a source of cedarwood oil and widely used in folk medicine (Seca et al., 2007; Gumral et al., 2015). In the present study, the chemical composition of the essential oils of two *Juniperus* species was investigated: *Juniperus horizontalis* Moench known as creeping juniper and *Juniperus communis* L. known as juniper berry cultivated in Egypt and the antiparasitic effect of the methanolic extract of these two species were investigated. Several biological activities has been tested for both species under investigation such as hepatoprotective (Manvi et al., 2010), anti-inflammatory (Tunon et al., 1995), analgesic (Banerjee et al., 2012), antibacterial (Sati et al., 2010; Eryiğit et al., 2014), anti-hypercholesterolemic (Akdogan et al., 2012), antioxidant Stoilova et al., 2014) and antimalarial (Milhau et al., 1997). These biological activities may be attributed to the

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diversity of chemical constituents found in this two species e.g. volatile oils (Hoferl et al., 2014; Chandra et al., 2007), flavonoids (Ilyas et al., 1990; Lamer 1975) glycosides, sterols and di and triterpenes (Souravh et al., 2014). One of the biggest challenges in Egypt is the control of bilharzias or Schistosomiasis. Schistosomiasis is a parasitic disease caused by the digenetic trematodes of the genus *Schistosoma* which are commonly known as blood flukes. It is well documented that *Schistosoma haematobium* was endemic in Ancient Egypt. It was first diagnosed in mummies by Ruffer in 1910 (Rashida, 2013). Schistosomiasis comes after malaria among parasitic diseases with regard to the number of people infected and those at risk of infection (Chitsulo et al., 2000). In the continuous search for a control of this parasitic infection, the total methanolic extract of the aerial parts of both *J. horizontalis* Moench and *J. communis* L were screened for their schistosomicidal and molluscicidal activities.

MATERIALS AND METHODS

Plant

Non-flowering aerial parts of *J. communis* L. and *J. horizontalis* Moench were collected on April 2013 from the International Garden at Cairo, Egypt. Identification of the plants was confirmed by Dr. Therese Labib, specialist of plant identification in El Orman Garden, Cairo, Egypt. Two voucher specimens (No. JH-36 *J. horizontalis* and JC-37 *J. communis*) were deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University.

Preparation of plant extracts and oil extraction

Fresh aerial parts of both *J. horizontalis* and *J. communis*, 200 g each, were suspended in twice their volumes of distilled water and subjected to steam distillation for 6-8 h using volatile oil distillation apparatus. Prior to distillation, the samples were chopped into about 2 cm long pieces. The distillate was allowed to cool at room temperature, volatile oil was allowed to separate from water and each essential oil sample was weighed on analytical scale (1.5 ml (0.75% v/w) from *J. horizontalis* and 1.7 ml (0.85 % v/w) from *J. communis*). The oils were collected, dried over anhydrous Na_2SO_4 and kept in a freezer at -5°C until the GC-MS analysis can be performed. 10 g of the concentrated total alcoholic extract of the aerial part of each of *J. horizontalis* and *J.communis* prepared in 80% methanol were reserved for schistosomicidal and molluscicidal study.

Schistosomicidal activity (WHO, 1985)

The schistosomicidal *in vitro* effect of each plant was determined according to Yousif et al. (2007). The schistosome material used in the present study was supplied by the Schistosome Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute, Cairo, Egypt. Adult male and female *Schistosoma mansoni* Sambon worms were collected 7 weeks post exposure of laboratory bred Syrian golden hamsters (*Mesocricetus auratus*) to cercariae by perfusion technique. Worms were cleaned from blood in small sieves of 20 µ mesh size using phosphate buffer (pH 7.4). A stock solution (500 µg/ml) of each plant extract was prepared in 100% dimethyl sulfoxide (DMSO) and 0.1 ml of this solution was made to 2 ml with RPMI 1640 containing 20% fetal calf serum, 300 mg streptomycin, cc 300 units pencillin and 160 µg gentamicin/100 ml medium. The obtained stock solution was diluted by the same medium to give the following concentrations: 100, 80, 60, 40 and 20 µg/ml. Three pairs of worms, males and females equally represented were placed in each well using sterilized forceps, they were exposed to these concentrations for 3 days and two replicates were done for each concentration. Praziquantel, the most effective schistosomicidal drug was used as a positive control (0.2 µg/ml) and a clean medium was used as a negative one to allow critical comparison of the effect. Test and control wells were maintained in an incubator at 37°C examined daily for 3 days for worm viability using stereomicroscope. Worms which did not show any sign of motility for one minute were considered dead. The activity of the plant extract was measured by calculating the number of dead worms relative to the total number of worms and compared with the negative (DMSO) and positive (praziquantel) controls.

For determination of LC_{50} (lethal concentration that kills 50% of the worms) and LC_{90} (lethal concentration that kills 90% of the worms), the same experiment was repeated six times. The worm mortality was recorded in each case and the LC50 and LC90 was determined using SPSS statistical program (version 20, Chicago, IL, USA).

Molluscicidal activity

The molluscicidal efficacy of the plant extracts was determined against the snails using standard method (WHO, 1965; El Bardicy et al., 2012). Adult *Biomphalaria alexandrina* (Ehrenberg) (Planorbidae) snails were obtained from the laboratory colony at the Schistosome Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute, Cairo, Egypt. A stock solution of 1 L of the dechlorinated water with a concentration 100 ppm of each extract was prepared and the following concentrations (20, 30, 40, 50 and 60 ppm) were tested and ten snails were added to each concentration. They were maintained in the solution for 24 h at room temperature (25 ± 1°C). After the exposure period, the snails were washed thoroughly with dechlorinated water and maintained in fresh water for another 24 h for recovery. In each case, two replicates were performed and two groups of snails were used as negative and positive control groups. The conventional molluscicide (niclosamide) at the same concentrations was used as a positive control; dead snails were counted in each case. Snails that were killed either during exposure or recovery period were counted and recorded.

For determination of LC molluscicidal effect, the same method using descending concentration was performed and LC_{50} and LC_{90} were determined by SPSS statistical program (version 20).

Chemical composition study

Gas chromatography/mass spectroscopy [GC/MS] analysis was performed using a Shimadzu GC-17A gas chromatograph equipped with a DB5-MS fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm) and coupled to GCMS-QP 5050 mass analyzer. Operating conditions were: carrier gas helium, flow rate 0.9 ml/min; oven temperature program: 40-240°C at 3°C/minute; sample injection port temperature, 240°C; detector temperature, 230°C; ionization voltage and ionization current were according to tuning result; scanning speed, 0.5 s; split, 1:54. Essential oil components peaks were first deconvoluted using AMDIS software. Compounds were identified by their Kovates indices (KI) relative to n-alkanes (C6-C20) and through matching mass spectra and retention indices with those deposited in the NIST, WILEY library database and

Table 1. Results of screening of schistosomicidal activity of total alcoholic extracts of both *J.communis* and *J. horizontalis* on *schistosoma mansoni worms*.

Parameter	*J. communis*	*J. horizontalis*	Praziquantel
LC_{50} (µg/ml)	91.4	91.7	0.27
LC_{90} (µg/ml)	127.4	143.2	0.37

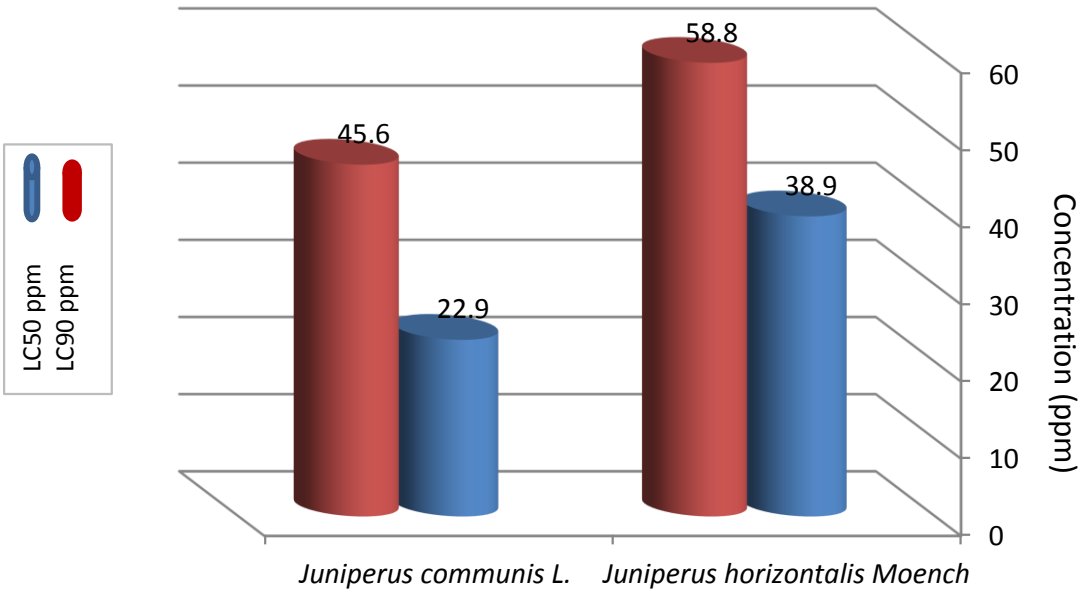

Figure 1. Results of screening of the molluscicidal activity of the methanolic extracts of both *J. horizontalis* and *J. communis.* Y-axis represent the concentration in ppm, X-axis represent LC_{50} and LC_{90} (% mortality) for different test fractions

reported in the literature. The distillation apparatus used was (VWR Scientific, catalog no.26319-008) rotatory evaporator (Buchi, G. Switzerland).

RESULTS

The screening results of schistosomicidal and molluscicidal activities of the total methanolic extracts of the two *Juniperus* species under investigation showed nearly equivalent schistosomicidal activity after 3 days exposure (Table 1). However this activity is less than that of praziquantel used as a positive control but still under 100 ppm. As for the molluscicidal activity against *Biomphalaria alexandrina* snails, *J. communis* extract showed higher activity with an LC50 equivalent to 22.9 ppm as compared to 38.9 ppm after one day exposure for *J. horizontalis* (Figure 1).

The chemical composition of the oils of the two *Juniperus* species under investigation analyzed by GC/MS (Table 2) showed a total of 60 compounds for *J. horizontalis* oil and 67 components for *J. communis* oil. The percentage of component identification for both plants was more than 90%.

DISCUSSION

Screening of the biological activity of the methanolic extracts of the two *Juniperus* species under investigation (Table 1 and Figure 1), showed that the schistosomicidal activity against *Schistosoma monsoni* worms of the total alcoholic extract of both species is nearly equivalent as seen in their LC_{50} values which is equal to 91.4 and 91.6 µg/ml for *J. communis* and *J. horizontalis*, respectively. In both cases, it is less than 100 ppm and consequently considered to posses schistosomicidal activity according to the recommendation of the world health organization (WHO, 1985). However, their effect is lesser than that of praziquantel (standard positive control used) showing an LC_{50} equal to 0.27 µg/ml. In the case of molluscicidal activity against *B. alexandrina* snails, *J. communis* extract showed higher activity than that of *J. horizontalis* with an LC_{50} = 22.9 ppm. These results are similar to that of

Table 2. Chemical composition of *J. communis and J. horizontalis* oils analyzed using GC/MS.

Identified compound	KI	*J. communis*	*J. horizontalis*
		area %	area %
1-Butene, 2-ethyl-3-methyl-	859.3	…	0.08
Tricyclene	924.7	0.46	0.4
α-Thujene	927.7	0.38	
α-Pinene	936.2	0.81	0.73
Vinylsulfonamide	946.3	0.25	….
Camphene	953.7	0.53	0.42
Sabinene	976.3	4.5	5.51
β-Pinene	981.9	0.19	….
β-Myrcene	990.4	5.15	5.94
Cyclobutane, 1,2-diethenyl-3,4-dimethyl	1002.1	0.21	….
N-Acrylonitrylaziridine	1002.2	…	0.03
α-Terpinene	1020.5	0.7	0.41
o-Cymene	1028.3	…	0.07
p-Cymene	1028.4	0.16	….
D-Limonene	1034	3.46	3.21
Bicyclo[4.1.0]heptane, 7-methylene	1048.1	…	0.14
Spiro[4.4]non-1-ene	1048.2	0.07	…
γ-Terpinene	1062.9	1.14	0.84
4-thujanol	1078.2	0.69	0.34
2,4(8)-p-Menthadiene	1090.5	…	0.55
α- Terpinolene	1090.7	0.62	…
Linalool	1102.1	0.61	0.25
2-Dimethylamino-3-methylpyridine	1109.5	…	0.15
Cyclopenta[c]pyran-1,3-dione, 4,4a,5,6-tetrahydro-4,7-dimethyl-	1110.6	0.19	…
6-Nonen-1-ol, (E)-	1114.9	0.5	…
Thujone	1126.3	1.98	4.8
β-Thujene	1133.3	…	0.2
α-Fenchene	1134	0.29	….
1H-Pyrrole-2-carbonitrile	1146	0.44	0.02
Butanenitrile	1148.3	…	0.11
Benzene, azido-	1149	0.15	…
α-Phellandrene	1151.7	…	0.18
(+)-2-Bornanone	1159.8	…	0.46
Camphor	1160.5	0.45	…
2-Fenchanol	1168.8	0.28	0.33
2-(1-Cyclopentenyl)furan	1179.2	…	0.39
2H-1-Benzopyran, 3,4-dihydro	1179.8	0.44	…
Ethanone, 1-(2-furanyl)	1184.2	…	0.17
5,5-Dimethyl-1,3-hexadiene	1184.9	0.2	…
Terpinen-4-ol	1191.1	3.82	2.88
Cyclohexanemethanol, α,α,4-trimethyl-	1204.2	…	0.29
α-Terpineol	1204.9	0.19	…
Urea, phenyl-	1218.4	…	0.16
2-Pyridinamine, N-nitro-	1219.2	0.05	…
Benzene, 1-methoxy-2-(1-methylethenyl	1226.9	0.92	1.01
1,6-Octadiene, 3,7-dimethyl-, (S)-	1229.7	…	0.21
1,6-Heptadiene, 3,3-dimethyl-	1231	0.41	…
1,2-Cyclooctadiene	1245.6	0.26	…
Methyl citronellate	1257.9	…	0.38
diazoadamantane	1259.5	0.45	…

Table 2. cont'd

cis-Geraniol	1265.7	…	0.08
4-Hepten-2-one, (E)-	1285.1	…	0.25
(Z) -8-Hydroxygeraniol	1286.4	0.41	…
Bornyl acetate	1295.4	…	41.17
Homogeraniol	1296.7	36.95	….
3-Phenylbut-1-ene	1316.6	…	0.05
Dispiro[2.2.2.2]deca-4,9-diene	1317.3	0.05	…
(E)-Geranic acid methyl ester	1324.1	0.09	0.09
1H-Pyrrole, 2,5-dihydro-	1330.1	…	0.07
Isobutyl 3-methylbut-3-enyl carbonate	1330.9	0.06	…
6,7-Dihydro-[1,2-e]-5H-pyrrolotetrazole	1357.8	…	0.06
Borane, ethylisopropylmethyl-	1358.6	0.09	….
5-Isopropenyl-1,2-dimethylcyclohex-2-enol	1382.2	…	0.08
4,4-Dimethyl-1,1a,3a,4,5,6-hexahydrocyclopropa[c]pentalene	1383.1	0.07	….
20-Carboethoxy-20-demethylvincadifformine	1397.2	…	0.1
2-Methoxycarbonylspiro[2.3]hexane	1398	0.14	…
3-Morpholino-1,2-propanediol	1401.4	…	0.15
Methanamine, N,N-difluoro-	1454.7	0.08	0.04
Silanamine, N-(dimethylsilyl)-1,1,1-trimethyl-, N-methyl	1467.5	0.07	…
4(3H)-Quinolinone, 3-hydroxy-	1481.6	0.04	…
D-Alanine, N-(4-butylbenzoyl)-, isohexyl ester	1488.1	…	0.14
Thiazole, 4-phenyl-	1489.1	0.14	…
(+)-Epi-Bicyclosesquiphellandrene	1501.3	…	0.15
cis-muurola-4(14),5-diene	1501.9	0.46	0.51
Tricyclo[6.2.1.0(2,6)]undeca-2(6),3-diene, 11-methyl-5,11-diaza-	1513.1	…	0.46
Cadina-3,9-diene	1531.2	…	1.68
Cadine-1,4-diene	1532.1	1.59	…
3-[Tetrahydro-3-thienyl]-2-oxazolidinone-S,S-dioxide	1537.8	…	0.08
Urea,1-furan-2-ylmethyl-3-[2-[(furan-2-ylmethylmethylamino) methyl]phenyl]-1-methyl-,	1538.6	0.1	…
N-(2-Phenylethenyl) acetamide	1547.3	…	0.06
10-Epi-elemol.	1563.5	9.45	8.44
3-Hexen-1-ol, benzoate, (Z)-	1581.9	0.1	…
9,9'-Bi-9H-fluorene, 9,9'-dimethoxy-	1588	0.08	…
1-Butyne, 3,3-dimethyl-	1598.3	0.24	…
6-(3-Methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol	1610.4	…	0.35
verbenyl acetate	1611.1	0.45	…
trans-β-Ionone	1624	2.25	1.93
1,3,4-Oxadiazole-2-thiol, 5-(3-pyridinyl)-	1633.8	0.28	…
α-Cubebene	1645.4	1.19	0.95
Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	1649.3	…	0.74
10-epi-γ-Eudesmol	1650.1	1.03	…
α-Cadinol	1659.2	…	1.96
(Cyclopropyl)trivinylsilane	1660.2	2.4	…
tau.-Cadinol	1672.1	….	2.41
tau.-Muurolol	1673.4	3	…
α-Eudesmol	1677.3	1.3	1.12
4-(Phenylmethyl)benzenemethanamine	1745.4	0.01	…
N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine	1779.6	0.03	…
Bergamotol, Z-α-trans-	1793.3	…	1.25
Lanceol, cis	1794	0.86	…
Ditrifluoromethyl(fluorocarbonyloxy)amine	1826.9	…	0.17

Table 2. cont'd

3,4-Dihydro-2,7-dimethylpyrimido[4,5-d]pyrimidine	1833.3	0.05	…
1,3-Di-n-Propyladamantane	1851.5	0.08	…
Ethanone, 1-(9-anthracenyl)-	1854.7	0.23	…
Acetamide, N-ethyl-N-(phenylmethyl	2888.4	…	0.19

previous study done on the genus *Juniperus* (*J. brevifolia*), showing high molluscicidal activity (Teixeira et al., 2012). Two other plants grown in Egypt belonging to family Cupressaceae were screened *in vivo* for schistosomicidal activity, namely *Chamaecyparis lawsoniane* and *Cupressus lasitanica* Mill. The extract of the bark of the former plant proved to show considerable effect with an LC50=59.6 ppm (Yousif et al., 2007).

It is worth mentioning that molluscicidal and schistosomicidal activity of several plants rich in volatile constituents were previously studied, such as *Thymus capitatus Marrubium vulgare* and *Chrysanthemum viscidehirtum*, showing promising activities against different parasites (Khallouki et al., 2000; Salama et al., 2012).

The oil content obtained by hydro-distillation of the aerial parts of *J. horizontalis* representing 0.75% v/w was analyzed using GC/MS (Table 2) showing a total of 60 compounds with a percentage of 95.39% of identified compounds. Bornyl acetate was seen as a major component representing 41.17% followed by 10-epi-elemol representing 8.44% and β-myrcene 5.94%, sabenine 5.51% and thujone 4.8%. This data greatly differs from that previously published on *J. horizontalis* essential oil composition (Eryiğit et al., 2014) and also differed from the composition stated by Cantrell et al. (2014) who showed that the major constituents present in the oil of *J. horizontalis* were alpha-pinene, sabinene and limonene. As for *J. communis* essential oil composition showed 67 component representing 0.85% v/w of which 1,7-Nonadien-4-ol, 4,8-dimethyl (homogeraniol) (36.95%) was the major component followed by 10-epielemol (9.45%) and β-Myrcene (5.15%), sabenine (4.5%), also this data differs from that already published on *J. communis* obtained from other sources mainly predominated by monoterpene constituents (Stoilova et al., 2014). Differences in essential oil composition may be expected due to geographical occurrences, climatic differences, the source of the oil either of commercial or of natural origin and may be due to the well proved relation between the time of collection and the percentage of active constituents and finally may be due to genetic variability among species (Khanzadi et al., 2015; Mammen et al., 2010). The high content of oxygenated compounds in the two species under investigation may explain the strong characteristic odor of these plants as referred to by Lund et al. (1981). However, Bornyl acetate was found to be absent in the aerial part of *J. communis*, while homogeraniol was found to be absent in the aerial part of *J. horizontalis*. It is worth mentioning that several compounds identified in the oil composition of the two studied species by the GC/MS analysis belongs to other classes such as hydrocarbons, fatty acids, minor alkaloids, amides and coumarins.

Conclusion

Both plants are good candidates for further studies on their schistosomicidal activity to determine the best effective doses to be used in the control of such dangerous parasite. The chemical composition of the essential oils of the two tested species of *Juniperus* differs greatly from the same species previously studied in different world zones and analyzed by similar analytical technique (GC/MS) which may be attributed to the difference in both climatic conditions and genetic characters of the studied species.

Conflict of interests

The authors have not declared any conflict of interests.

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Antioxidative and anti-hyperglycaemic effect of *calotropis procera* in alloxan induced diabetic rats

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This study investigated the anti-hyperglycaemic and antioxidative properties of *Calotropis procera* (Tumfafiya) leaf extract on an alloxan induced diabetic rats. The rats were fed on commercial diet and grouped into 4. Group1 as diabetic treated with leave extract (DTL), group 2 diabetic on normal diet (DNT), group 3 as non diabetic on normal diet (NDNT) and group 4 as diabetic treated with chlorpropamide (DCP). The successful diabetic induction was achieved by intra-peritoneal injection of 180 mg/kg body weight with alloxan. The sustenance and severity of diabetes was assessed using fasting blood glucose for one week. The plant extract was then administered to the induced rats at 100 mg/kg body for four weeks after which a serum glucose level was assessed at weakly intervals. Malondialdehyde was also measured for anti-oxidative effect. The results indicated that the extracts possessed significant hypoglycemic effect on the DTL group, with mean glucose of 5.9 ± 0.2 mmol/L compared to the DNT control group with a mean glucose of 15.5 ± 0.2 mmol/L ($p < 0.05$). A raised malondialdehyde was also observed among the DNT (23.2 ± 3.5) as against DTL group (12.5 ± 0.5) mmol/L, ($p < 0.05$). In conclusion, it shows that C*alotropis procera* methanolic leaf extract have a potential hypoglycemic effect in alloxan induced diabetic rats and also with antioxidant property.

Key words: *Colotropis procera*, anti-hyperglycaemic agent, antioxidant, alloxan, diabetes mellitus, chlopropamide.

INTRODUCTION

Diabetes mellitus is a metabolism disorder characterized by inappropriate hyperglycemia (Ceriello, 2005), caused by a relative or absolute deficiency of insulin or by resistance to the action of insulin at the cellular level (Bakari and Narayan, 2003; Fasanmade et al, 2008). It is the most common endocrine disorder, affecting as many as 200 million people worldwide (Debra, 1991; Brown et al., 2003). The clinical consequences of the syndrome include blindness, heart and blood vessel disease, stroke, kidney failure, amputation and nerve damage and with up to 80% of death in people with diabetes caused by cardiovascular in the developed world (Cariello and Motz, 2004). *Colotropis procera* belongs to the family Asclepiadaceae with various nomenclatures as Sodom

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apple, auricular tree, deadsea apple, swallow-worth, giant-milk weed, madar, mudar, rubber bush, small crown flower, sodom's-milk weed, from different ethnic or geographical locations (Ansari and Ali, 1999; Aliyu et al., 2006). In Hausaland, it is called Tunfafiya or Bambambele and found on dry savannah and other arid areas, mostly anthropogenic occurring around villages (Aliyu et al., 2006). *C. procera* is reported to contain cardenolides besides steroids and triterpenes (Ansari and Ali, 1999; Shrivastava et al, 2013). From the hexane-insoluble fraction of this medically important plant, a new cardenolide named proceragenin was isolated. Pharmacological screening of proceragenin revealed to poses antibacterial and anti-aggregating activities (Akhtar, 1984; Uddin et al, 2012).

There is a reservoir of basic information on the ethno medicinal uses of *C. procera* in the treatment of headache, catarrh, conjunctivitis, skin disease and wound (Vishwa, 2004). Other uses include treatment against asthma, cold, cough, labor, leprosy, parturition, small pox, sore, splenitis, purgative, fatality, fumitory, dyspepsia (Duke, 1994; Kawo et al., 2013). However, there is paucity of information as an anti-diabetic agent though used by herbalist in Hausaland, in the management of diabetic patients. The present research was conducted to study the potential hypoglycaemic and anti-oxidative activities of the leaf extract of *C. procera* in alloxan induced diabetes rats. An extension was also made to provide an introductory approach for the evaluation of its traditional preparation in order to scientifically validate the therapeutic preparation of this plant in the control of diabetes in our locality.

MATERIALS and METHODS

All the chemicals and reagents used are of analytical grade and were obtained from Sigma-Aldrich (St Louis, USA).

Plant collection

The plants of *C. procera* as a whole were collected from natural populations of the Bayero University, Kano. The plant was authenticated and identified at the Botanical Unit of the Department of Biological Science. The plant material was shade dried at room temperature for 10 days, coarsely powdered with the help of a hand grinding mill, and the powder was passed through sieve.

Phytochemical sreening

Phytochemical screening of the leaf extracts was carried out following the standard methods prescribed by Sofowara in 1993 and Trease and Evans, 1989 evaluate the presence of various chemical constituents in ethanolic leaf extracts.

Experimental animals

White albino rats were obtained from the animal house of Physiology Department, Bayero University Kano, where the animals were kept for the period of study.

Plant extract preparation

C. procera leaf was collected and dried under shade for 7 days. The dried leaves were grounded into powder manually and processed to mill. About 750 g of the powdered leaf was added to a container with 1,500 ml methanol and allowed to stand for 8 days with occasional swirling and shaking. The content was then evaporated using rotator evaporator for a solid leaf extract.

Extract administration

From the dried extract, one teaspoon of the powdered leaf extract weighing 8.98 g was mixed with the animal feed weighing 100 g. The mixed animal feed with the leaf extract was fed to the animals on daily basis for the period of four consecutive weeks. The treatment was stopped and the rats were sacrificed. During the entire four weeks of the experiment, fasting blood glucose of the rats was monitored at an interval of one week.

Induction of diabetes

All animals were fed on normal diet and left to acclimatize for a week. Diabetes was induced by a single injection of 100 mg/kg body weight alloxan monohydrate freshly prepared, through intraperitoneal (IP) route to fasting rats for at least 10 h. Blood glucose level were measured prior to induction and after the induction.

Animal feed

Commercially prepared rats feed (grower mash) was obtained from PS mandrides plc, Kano, Nigeria. The composition of the feed is given below:

Crude protein	15.50%
Crude fiber	7.40%
Calcium	6.59%
Phosphorus	4.85%
Metabolized energy cal/kg	2500k

Experimental design

A total of 12 Wistar (albino) rats were randomly grouped into four (4) groups as follows:

Group 1: Alloxan- induced diabetic rats administered treated with mixed feed (DLT).
Group 2: Negative control group fed with normal animal feed (NDNT).
Group 3: Positive control group fed with normal animal feed (DNT).
Group 4: Diabetic treated with Chlorpropamide (DTCP).

Sample collection

Weekly samples for the estimation of glucose was obtained aseptically and treated accordingly to guard against lost on storage. At the end of the four weeks, the rats were sacrificed by surgical dislocation of the neck and the blood was collected into test tubes and centrifuged to get the serum for the biochemical analysis. The Serum was used for the estimation of glucose (fasting blood sugar) and malondialdehyde (MDA).

Table 1. Phytochemical screening of crude leaf extract *Calotropis procera*

Chemical constituents	Methanol
Alkaloids	+
Terpenoids	+
Flavonoids	+
Anthraquinones	-
Tannins	+
Saponins	+
Glycosides	+
Reducing sugars	-
Steroids	+

Table 2. Fasting blood sugar (mmol/l) of alloxan induced diabetic rats treated with *calotropis procera* leave extracts.

Parameter	Serum glucose level (mmol/L) at induction and post treatment			
	Leaf (Ext)	Controls		CP (8.4 mg/kg)
Week	DTL	DNT	NDNT	DTCP
1 (ind)	13.95±1.26	**1**2.85±0.36	4.32±0.22	12.90±0.20
2	10.93±0.47	13.79±0.31	4.16±0.14	10.44±0.08
3	8.07±0.48	13.12±0.16	5.48±0.32	8.99±0.09
4	6.42±0.58	14.29±0.32	3.79±0.08	8.27±0.13
5	**5**.91±0.17	14.51±0.18	4.33±0.13	6.06±0.07

DTL=diabetic treated; DNT=Diabetic non treated; NDNT=Non-diabetic non treated; ind=induction week. Values are mean ± standard error of mean (n = 12); CP= Chlorpropamide.

Biochemical analysis

Serum glucose estimation using glucose oxidase method

Fasting blood glucose was estimated by glucose oxidase method according to manufacturer's instruction (Erba diagnostics, Mannhem, Gmbh Germany).

Determination of malondialdehyde (MDA) (Gutterridge and Wilkins, 1982)

Malondialdehyde is an organic compound with the formula $CH_2(CHO)_2$. The structure of this species is more complex than this formula suggest. The reactive species occurs naturally and is a marker for oxidative stress. Lipid peroxidation generates peroxide intermediates which upon cleavage releases MDA a product which react with thiobarbituric acid (TBA). The product of the reaction is a colored complex which absorbs light at 532 nm.

RESULTS

Phytochemical screening

Phyto-chemical screening of the crude extract of *C. procera* leaf was carried out to ascertain the presence of its bioactive constituents utilizing standard methods.

Mean serum glucose level during extract administration

There was a decrease in mean serum glucose level (mmol/L) of the rats from the first to the fourth week of plant extract administration. Tables 1 to 3.

Mean malonyldialdehyde concentrations among alloxan induced diabetic and controls

Malonyldialdehyde concentration was estimated at the end of four weeks post induction. As compared, there was an observed significant increase among DNT group than any other group in the study.

DISCUSSION

Diabetic mellitus is a metabolic disease associated with

Table 3. Malonyldialdehyde (mmol/L) concentrations among alloxan induced diabetic rats and controls.

Groups	Malondialdehyde (mmol/L)
DT L	12.5±0.46†
DNT	23.2±3.46
DTCP	25.2±5.65
NDNT	8.2±0.55

DTL=diabetic treated; NDNT=non-diabetic non-treated; DNT=diabetic untreated; DTCP=Diabetic treated with Chlorpropamide. Values are mean ± standard error of mean (n = 12), †=significant.

impaired glucose metabolism which in effect alters intermediary metabolism of lipids, of which most of the complications of the diabetic state are initiated by the generation of free radicals (Kumar et al., 2005). Despite immense technological advancement in modern medicine, many people in developing countries still depend on traditional healing practices and medicinal plants for their daily health care needs. There exists a good virtue to intensify researches into plants for medicine, especially those that will reduce the burden in serious disorders, such as diabetes mellitus. In trado-medical practice by herbalists, it is believed that *C. procera* is used locally for the treatment of diabetes mellitus. Phytochemical studies unveil that extract of *C. procera* contains terpenoids, steroids, glycosides, saponins, alkaloids and flavonoids similar to the work reported by Verma et al. in 2013 and Kawo et al. in 2013; Shrivastava et al 2013. Minerals such as magnesium, manganese, zinc, iron, phosphorous, copper and calcium among others were reported in the work of Vishwa (2014). The study as highlighted by the presence of many secondary metabolites provides an overview of the different classes of molecules present that have led to their pharmacological activities. There was observed in this research work, a significant reduction in mean serum glucose of the rat treated with the plant leaf extract (5.91 ± 0.17) when compared with the mean serum glucose of the diabetic control rats (14.51 ± 0.18) ($p < 0.05$). The hypoglycemic effect of the plant extracts was maintained throughout the period of administration. The detection of flavonoids in the extract might be a pivot to the blood glucose lowering property. Flavonoid is believed to inhibit glucose-6-phophatase activity in a liver, thereby suppressing gluconeogenesis and glycogenolysis and consequently reduces the hyperglycaemia. This can therefore be the reason behind the progressive decrease in the antidiabetogenic effect of the extract among DLT group from induction down the 4th week. Some plants extracts with similar elemental and organic constituents have same biochemical influence on glycaemic control as reported by Rajendran et al. in 2007 on *Aloe vera* with a wide range of medicinal applications such as lowering blood sugar in diabetes, ulcer curative effect, stimulating immune response against cancer etc. These effects are being attributed to the role of inorganic elements like zinc, iron, copper, magnesium and manganese in collaboration with flavonoids and alkaloids in the improvement of impaired glucose tolerance. The concentration of the MDA across the set groups were significantly higher in DNT (23.2 ± 3.46) and DTCP (25.2 ± 5.65) compared to that of DTL (12.5 ± 0.46) and NDNT (8.2 ± 0.55) ($p < 0.005$) groups statistically.

Malondialdehyde is a degradative product of peroxidation of polyunsaturated fatty acids (PUPA) in the cells membrane (Ohakawa et al., 1979). The presence of MDA in circulation indicates oxidative stress which has been reported as one of the underlying cause of hyperglycaemia. *C. procera* possesses potent co-factors among the minerals reported by Vishwa in 2014, for a number of antioxidants by increasing levels of endogenous antioxidants, viz. superoxide dismutase, catalase and glutathione and bring down the level of thiobarbituratic acid-reactive substance and anti-hyperglycemic effects.

CONCLUSION

Significant number of researches have shown the presence of a wide variety of bioactive compounds in the leaf, stem and roots of medicinal plants including *C. procera* that have beneficial effects on human health. Considering the fact that diabetic mellitus have reached epidemic proportions in many countries with increase in socio-economic burden, it will be important to have an alternative that will help in prevention and treatment of this disease. The studies using *C. procera* as a medicinal plant bring information that may provide validation for its medicinal uses. A more extensive research involving clinical trials is requested so that it could be recommended for prevention and as an adjuvant in the treatment of diabetic mellitus and its complication.

RECOMMENDATIONS

As part of the efforts in evaluating the anti-hyperglycemic effects of leaf extract of *C. procera,* It is recommended that the histology of the liver cell be carried out so as to have the physical observation of the toxicity in liver. Also, the antioxidant effect should be assayed by using other oxidative stress marker, such as super oxide dismutase, glutathione peroxidase, etc to confirm the antioxidant effect.

Conflict of interest

The authors have not declared any conflict of interest.

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