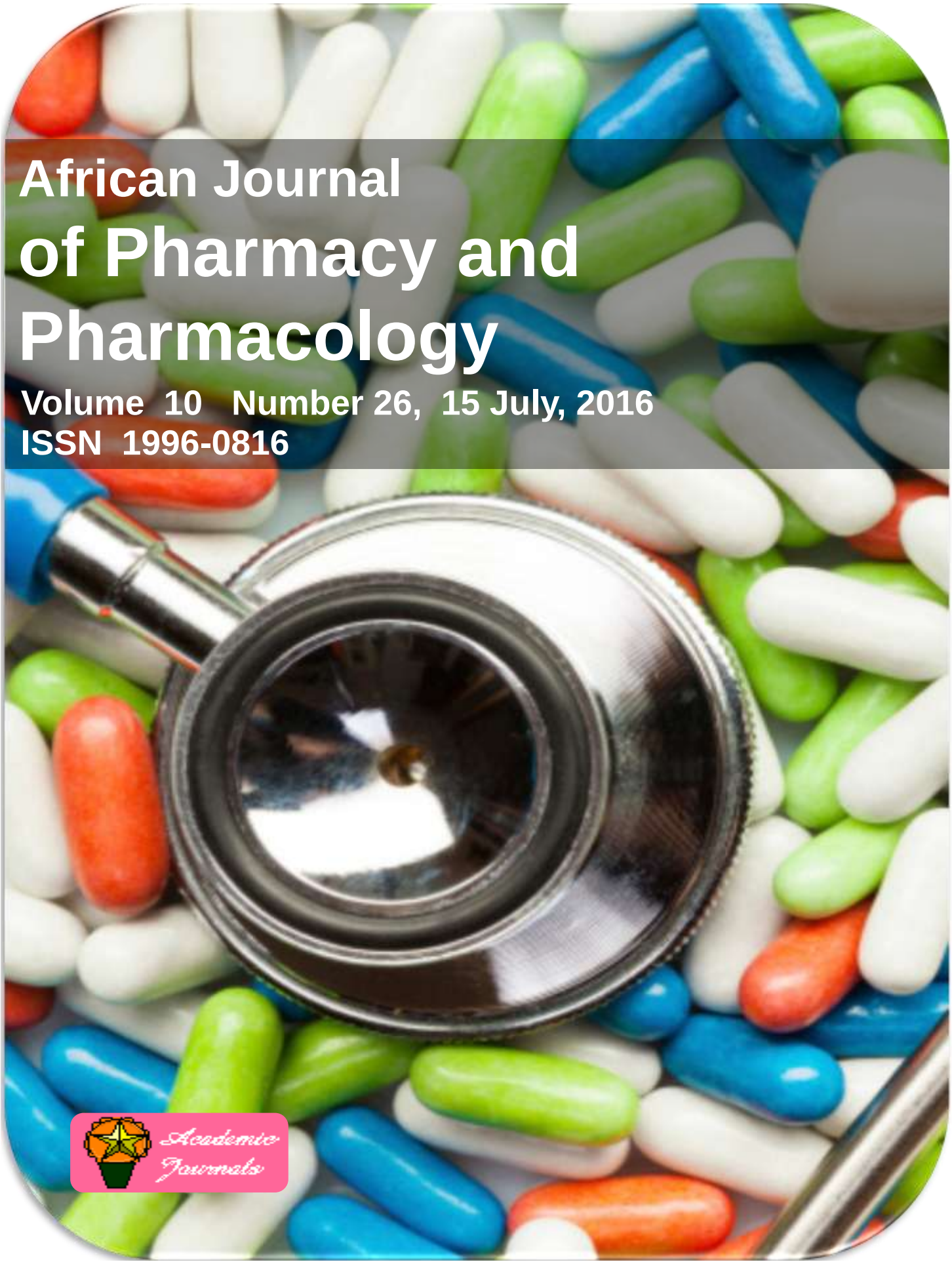

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Full Length Research Paper

Centratherum anthelminticum seeds reverse the carbon tetrachloride-induced hepatotoxicity in rats

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The present study is the first attempt to evaluate the hepatoprotective effect of ethanolic seeds extract (ESEt) of *Centratherum anthelminticum* (black cumin) in carbon tetrachloride (CCl_4)-induced liver injury. The test doses (600 and 800 mg/kg) of same extract were found effective in their respective test groups by improving the body and liver weights, serum alanine and aspartate transaminases, γ-glutamyltranspeptidase, alkaline phosphatase, total proteins, albumin, total bilirubin, especially indirect bilirubin and uric acid levels as compared to CCl_4-induced hepatotoxic control group. In addition, decreased percent inhibitions of antioxidant parameters including catalase, superoxide dismutase and reduced glutathione accompanied with increased percent inhibition of lipid peroxidation observed in both test groups. Histopathological studies also proved the liver regenerating property of ESEt by showing decrease in fatty deposition, necrosis and inflammation around the central vein of liver lobules. Therefore, the ESEt was found to be hepatoprotective and antioxidative in nature.

Key words: *Centratherum anthelminticum*, Carbon tetrachloride, Liver function test and antioxidants.

INTRODUCTION

Liver is the indispensable organ in maintaining the homeostasis in body by regulating metabolic and hematological functions plus bile production for fats emulsification and digestion (Hall, 2011). Besides these, diverse functions are also on its credit like storage of vitamins, iron, detoxification, removal and excretion of antibiotics, hormones, oxidative radicals and xenobiotics (Hall, 2011). However, liver's function and structure are very sensitive and can easily be affected by microorganisms (bacteria/viruses/fungi/parasites) and hepatotoxins/carcinogens (Ahmad et al., 2014). Among different liver affecting chemicals, carbon tetrachloride (CCl_4), which is normally used as cleaning agent in industries, in fire extinguishers, etc., is injurious for body

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tissues especially liver. Continuous inhalation of CCl_4 vapours enhanced inflammation and necrosis of hepatocytes by producing reactive metabolites that may lead to severe cirrhosis, if the condition ignored for a long time (Bigoniya et al., 2009; Singh et al., 2011). Excluding genetic causes, environmental pollutants and chemicals used in industries are also accelerating the risk of liver problems in both developed and developing countries and Pakistan is also showing highest burden of liver disorders which is chiefly contributed by mines, mills and industrial workers (Anjum et al., 2009; Malaguarnera et al., 2012; Shah et al., 2015).

In addition to the costly conventional treatments of liver problems, medicinal plants always have prominent place in this regard especially in Asian countries as these are easily available and have no toxic effects (Guan and He, 2013). *Centratherum anthelminticum (Vernonia anthelminticum* Willd) belongs to the family Asteraceae and commonly known as black cumin (Amir and Chin, 2011). It is not only widely distributed in neighboring countries of Pakistan but also popular for its culinary use in all over India and Pakistan (Singh et al., 2012). Its bitter taste seeds are popular for various medicinal purposes and their different extracts are well-reported for pharmacological activities like analgesic, antipyretic, antimicrobial, anticancer, antidiabetic, antihyperlipidemic, anti-inflammatory, antioxidant, antiurolithiatic, and skin problems (Amir and Chin, 2011; Mudassir and Qureshi, 2015). However, their hepatoprotective effect has not been documented yet. Therefore, the present study is designed to investigate the effect of ethanolic seeds extract of *C. anthelminticum* in carbon tetrachloride (CCl_4) induced hepatotoxic rats.

MATERIALS AND METHODS

Animals

Female Wister albino rats (180 to 220 g) were purchased from breeding house of Dow University of Health Sciences (DUHS), Karachi and kept in animal house of Department of Biochemistry, University of Karachi (UoK) according to the international guidelines of animal handling by giving them standard laboratory diet and water *ad libitum*.

Plant material and extraction

Seeds of *C. anthelminticum* were purchased and identified by authentic taxonomist (voucher no. KU/BCH/SAQ/02) of UoK. These seeds were used to prepare ethanolic extract (ESEt) according to the procedure described by Mudassir and Qureshi (2015).

Positive control and vehicle

Silimyrin (Siliver 200 mg) and dimethyl sulphoxide (DMSO; 0.05%) of Abbott Laboratories (Pakistan) Ltd and Fischer Scientific, UK, respectively were used as positive control in the present study and vehicle for administering the doses of ESEt in experimental rats.

Animal grouping and procedure

Experimental rats were divided into four groups (6 rats/group) including normal and hepatotoxic controls (group I and II), each of them treated with distilled water (1 ml/kg), positive control (group III) treated with silymarin (200 mg/kg) and test groups (groups IV and V) treated with ESEt (600 and 800 mg/kg). Each treatment was done orally once per day in early morning for 5 days consecutively. However, hepatotoxicity was induced in group II to V by intra-peritoneal injection of CCl4 (3 ml/kg in 1:1 ratio with olive oil) on 3rd and 5th day of trial after 1 h of their respective treatments. After 24 h of last dose of CCl4, rats were sacrificed to collect blood and serum to analyze biochemical parameters. In addition, livers were dissected out carefully to weigh them, estimate antioxidant parameters and for histological examination. The present experimental procedure was approved by Board of Advance Study and Research (BASR) of UoK.

Physical, biochemical and antioxidant parameters

Percent body weight change (BWC) of rats in each group was calculated by using formula (Azmi and Qureshi, 2013) after measuring the body weights of each rat on initial (IBW) and final (FBW) days of trial. Beside this, the livers of each group were also weighed as LW (g).

$$Percent\ BWC = \left(\frac{FBW - IBW}{IBW}\right) X\ 100$$

Biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), total bilirubin (TBR), direct bilirubin (DBR), indirect bilirubin (IBR), total protein (TP), albumin (ALB) and uric acid (UA) were measured in serum through commercially available enzymatic kits (Randox, UK). Whereas antioxidant parameters including, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and lipid per oxidation (LPO) were measured in liver homogenate by standard manual methods (Lateef and Qureshi, 2013). Percent protection in ALT, AST, and ALP of positive control and test groups against hepatotoxic control was calculated with the help of following formula (Al-Qarawi et al., 2004) where At, Ax and Ao are the mean readings of group III/IV/V, II and I of respective enzyme.

$$Percent\ Protection = 1 - \left(\frac{At - Ao}{Ax - Ao}\right) X\ 100$$

Similarly, percent gain/loss in LW and TBR, IDBR, TP, ALB and UA levels in positive and test groups was calculated with formula described by Azmi and Qureshi (2012).

HISTOLOGY

Dissected out liver tissues from each group were immersed in 10% formaldehyde solution separately and sent to Dr. Essa's diagnostic laboratory, AbulHasan Isfahani Road, Karachi Pakistan for histological studies.

Statistical analysis

One way analysis of variance (ANOVA) was used to analyze the results of present study and mentioned as mean ± SD (standard deviation). The difference of mean of each parameter was compared among all groups and found significant at $p<0.05$ through

Table 1. Effect of ESEt on physical and biochemical parameters.

Groups	LW (gm)	GGT (U/l)	TBR (mg/dl)	DBR (mg/dl)	IBR (mg/dl)	TP (gm/dl)	ALB (g/dl)	UA (mg/dl)
I: Normal Control	7.46±1.23	10.0 ± 0.0	0.23 ± 0.3	0.10 ± 0.01	0.0 ± 0.0	5.52 ± 0.20	4.46 ± 0.24	8.51 ± 0.21
II: Hepatotoxic Control	15.31±2.06	8.6 ± 6.3	0.43 ± 0.22	0.2 ± 0.16	0.38 ± 0.21	3.70 ± 0.37	2.91 ± 0.11	10.20 ± 0.34
III: Positive Control	11.93±1.19 ^{c} (-22%)	0.71 ± 0.47^{c}	0.19 ± 0.01^{b} (-55.8%)	0.10 ± 0.01	0.10 ± 0.008 (-73.6%)	5.26 ± 0.19^{c} (52%)	4.10 ± 0.04 (41%)	8.86 ± 0.12^{c} (-13.13%)
IV: Test group	11.30±1.43 ^{c} (-26.1%)	1.36 ± 0.32^{c}	0.20 ± 0.01^{b} (-53.4%)	0.10 ±0.009	0.10 ± 0.007 (-73.6%)	5.10 ± 0.08^{c} (37%)	4.35 ± 0.29^{c} (49%)	9.17 ± 0.55^{c} (-11.2%)
V: Test group	10.60±1.07 ^{c} (-30.7%)	3.05 ± 0.08^{c}	0.26 ± 0.01 (-39.5%)	0.19 ± 0.009	0.10 ± 0.009 (-73.6%)	5.90 ± 0.08^{c} (59%)	4.56 ± 0.06^{c} (56%)	7.60 ± 0.64^{c} (-25.4%)

Each value represents the mean ± SD (n=6). b & c = p<0.01 & p<0.0001 when compared with group II. Values in parenthesis represent the percent gain (+) / loss (-) in parameters.

least significance (LSD) test (SPSS, version 17.0).

RESULTS

Effect of ESEt on percent body weight change and liver weights

ESEt in doses of 600 and 800 mg/kg significantly decreased (p<0.05 and p<0.0001) the percent reduction in body weights in group IV and V, respectively as compared to hepatotoxic control group (group II) where administration of CCl_4-induced marked reduction in body weights (Figure 1). Similarly, the liver weights (g) in group II were clearly increased (p<0.0001) as compared to group III, IV and V where silymarin and test doses of ESEt were found effective in normalizing the weights of livers (Table 1).

Effect of ESEt on liver and non liver-specific parameters

Liver-specific enzymes including ALT, AST and ALP (U/l) were drastically elevated in group II whereas all same enzymes were significantly decreased (p<0.01 and p<0.0001) in groups III, IV and V which were treated with silymarin (100 mg/kg) and ESEt (600 and 800 mg/kg), respectively and showed significant percent protection especially in ALT (95 to 98%), AST (73 to 81%) and ALP (37 to 100%) (Figure 2). Whereas GGT (U/l) level was found decreased in group II (Table 1). Similarly, ESEt and silymarin were found decreasing TBR level especially indirect BR (mg/dl) in their respective groups as compared to group II (Table 1). On the other hand, TP (g/dl) and ALB (g/dl) were decreased in group II and became significantly increased (p<0.01 and p<0.0001) in group III, IV and V (Table 1). Beside these, non-liver-specific parameter uric acid (mg/dl) was found decreased prominently (p<0.0001) in the last three groups (Table 1).

Effect of ESEt on antioxidant parameters

Percent inhibitions of CAT, SOD and GSH were significantly decreased in positive control (group III) and test groups (IV and V) as compared to hepatotoxic control group II (Figure 3 and 4). Whereas, in the case of percent inhibition of LPO, an entirely opposite picture was observed in positive and test groups (Figure 4).

Effect of ESEt on histology of liver tissues

Histopathological studies were done by preparing slides of liver tissues stained with hematoxylin and eosin (Figure 5). Liver tissue (slide A) of CCl_4-induced hepatotoxic group showed degeneration of hepatocytes accompanied with fatty deposition (ballooning) and infiltration of inflammatory cells around dilated central vein in liver lobule. However, all these features of liver injury were gradually recovered in tissues (slide C and D) of groups treated with ESEt in doses of 600 and 800 mg/kg.

DISCUSSION

Liver problems, because of acquired causes, are contributing the major portion of death burden globally (Malaguarnera et al., 2012). The characteristic features of liver problems are loss of appetite and weight, elevation in liver-specific parameters and reduction in hepatic functions (Singh et al., 2011). Interesting, the same features was clearly observed in the present carbon tetrachloride (CCl_4)-induced hepatotoxic rat model. CCl_4 is a well-known liver toxicant (Adewale et al., 2014). It severely damaged cellular integrity of hepatocytes by converting itself into reactive metabolites including trichloromethyl ($.CCl_3$) and peroxytrichloromethyl ($.OOCCl_3$) radicals after passing through hepatic cytochrome P_{450} enzyme responsible for the detoxification of xenobiotic or chemicals (Khan et al., 2012).

Elevated levels of both ALT and AST are the best indicators of liver damage whereas GGT and

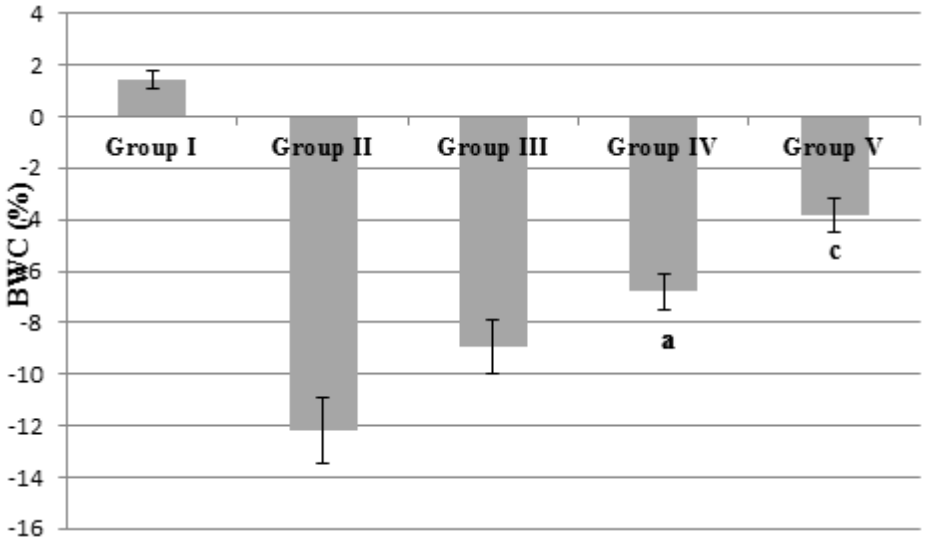

Figure 1. Effect of ESEt on Percent BWC. Each bar represents the mean ± SD (n=6). a and c = p<0.05 and p<0.0001 respectively when compared with group II.

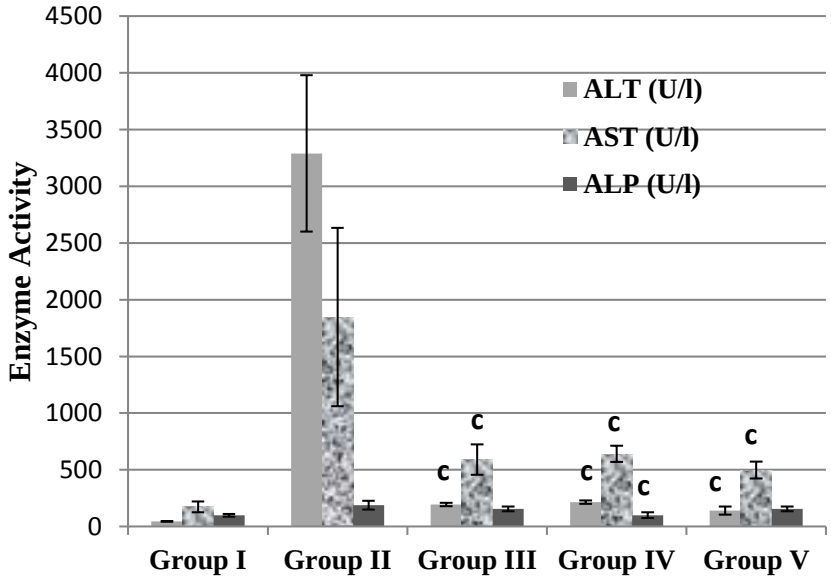

Figure 2. Effect of ESEt on Liver-Specific Enzymes. Each bar represents the mean ± SD (n=6). c = p<0.0001 when compared with group II.

ALP together reflect the damage in bile duct. However, isolated elevation of AST and ALP is the warning of cardiac and bone problems as their chief concentrations are present in both these tissues, respectively (Bishop et al., 2013). In the present study, CCl_4-induced hepatotoxic control rats showed a drastic increased in ALT, AST, ALP and decreased in GGT levels. On the other hand, ALT, AST and ALP were found extensively improved in positive control and test groups treated with silymarin (100 mg/kg) and two doses of ESEt (600 and 800 mg/kg) while GGT remained low or below normal in these groups. ALT, AST and ALP are the intracellular enzymes and their abundance presence in serum above 100 to 1000 folds of their normal levels is the echo of the alteration in cell membrane intactness (Bishop et al., 2013) while decreased levels of GGT are the reflection of intrahepatic cholestasis and decreased bile acid production (Hyder et al., 2013). The alteration in cell

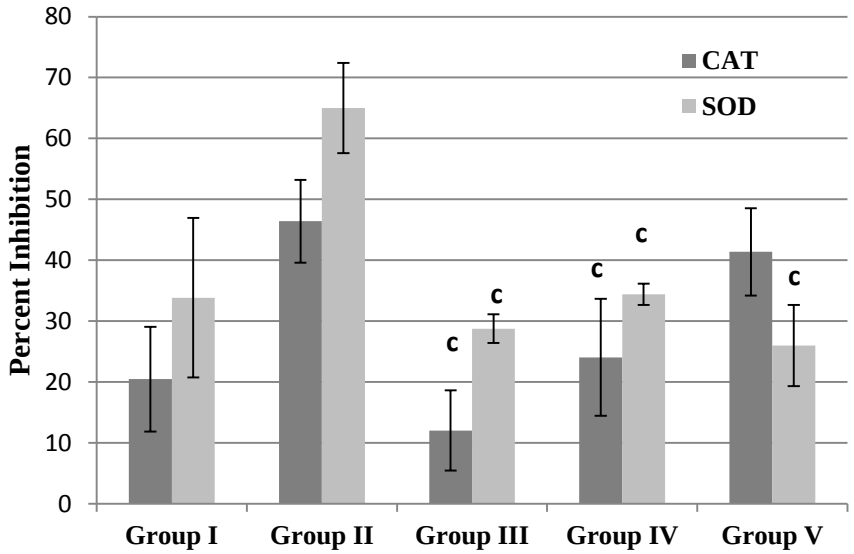

Figure 3. Effect of ESEt on Percent Inhibition of CAT and SOD. Each bar represents the mean ± SD (n=6). c = p<0.0001 when compared with group II.

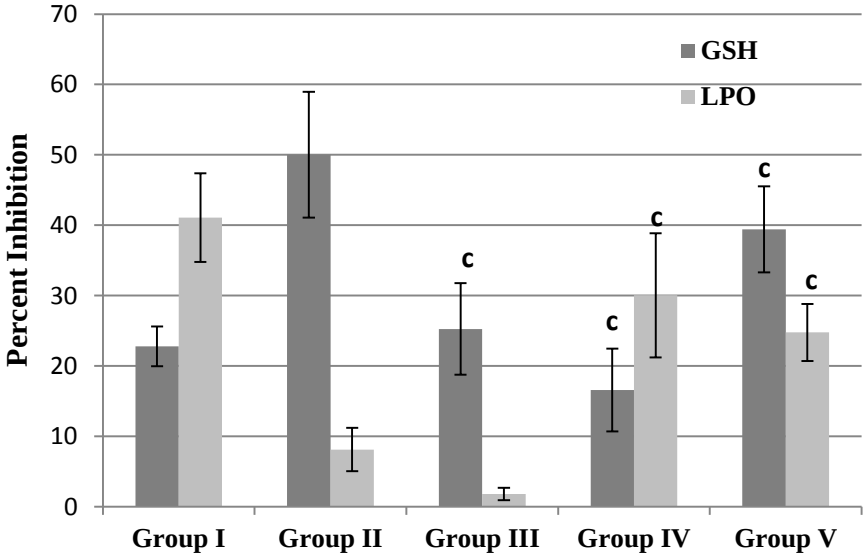

Figure 4. Effect of ESEt on Percent Inhibition of GSH and LPO. Each bar represents the mean ± SD (n=6). c = p<0.0001 when compared with group II.

membrane was clearly proved by observing severe necrosis along with inflammation and steatosis (fatty deposition) in hepatocytes around central vein of lobules in microscopic examination of liver tissues of hepatotoxic group which was gradually healed up and improved by observing almost normal architecture of liver tissues that were dissected out from test groups treated with both doses of ESEt, respectively. Important point is that the ESEt of *C. anthelminticum* showed much better results as compared to silymarin in bringing the liver anatomy back to normal.

The cellular protective effect of ESEt of *C. anthelminticum* was also confirmed by observing decreased levels of uric acid (UA) in both test groups as compared to hepatotoxic control group which showed high concentration of same parameter. UA is the end product of purine metabolism and its abnormally increased concentration in serum is the alarming sign not only for the presence of kidney dysfunction but also for the degradation of body tissues or cells (Kutzing and

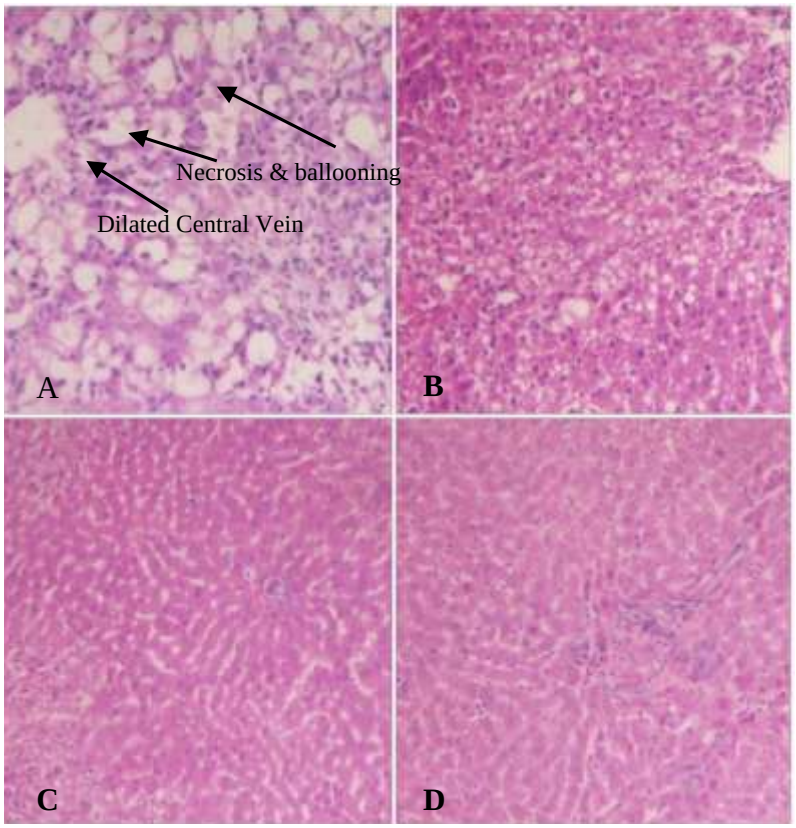

Figure 5. Effect of ESEt on Liver Tissue. A= CCl_4-induced hepatotoxic control group that showed fatty deposition (ballooning), necrosis and inflammation around central vein. These toxic features are greatly improved in test groups treated with ESEt @ 600mg and 800mg/kg (C & D). However, inflammation and ballooning can be observed in liver slide of silymarin (100mg/kg) treated group (B).

Firestein, 2008). This beneficial effect of ESEt was more strengthen by observing a significant decrease in percent reduction in body weights of both test groups treated with same extract (600 and 800 mg/kg) as compared to hepatotoxic control which was only treated with CCl_4 (3 ml/kg) and showed an extreme percent loss in body weight up to -12%. Even silymarin, the well-known hepatoprotective medicine, did not prove to be statistically efficient in this respect in positive control group.

The possible involvement of ESEt of *C. anthelminticum* in regenerating liver tissue was also fortified by estimating the normal levels of total protein (TP), albumin (ALB) and total bilirubin (TBR) both direct and indirect in extract treated test groups as compared to CCl_4-induced hepatotoxic control group that showed decreased levels of TP, ALB and elevated levels of TBR particularly indirect/ unconjugated one. Literature declared that 90% of total protein, except immunoglobulin and 100% albumin are synthesized in liver (Murphey et al., 2007). However, TP and ALB can also be decreased in renal functions and malnutrition but these are considered as the best meter for evaluating the functionality of liver (Thapa and Walia, 2007). Similarly, increased serum IDBR level was not only observed in severe hemolysis but also in liver problems including hepatitis and cirrhosis with decreased or no conjugation reaction taking place in liver (Hall, 2011). Another study stated that depletion of TP induced intense mitosis in hepatocytes which leads to the enlargement of liver and this condition persists till the TP concentration becomes normal in blood (Bishop et al., 2013; Hall, 2011). Interestingly, the same happened in the present study as decreased TP and ALB was observed in hepatotoxic control group accompanied with increased liver weights, whereas improvement in TP and ALB levels in positive control and test groups also normalized the liver weights in these groups. The observed liver regenerating ability of ESEt in the present study might be by inhibiting the factors that hindered liver tissue regeneration like tumor necrosis factor alpha (TNF-α), etc (Kang et al., 2012). Amazingly, chloroform fraction of *C. anthelminticum* seeds was claimed to inhibit TNF-α in human tumor cells (Arya et al., 2012).

The antioxidant property of ESEt of *C. anthelminticum*

was already well-reported in hyperlipidemic rabbits (Lateef and Qureshi, 2013) and again became beneficial in contributing to the hepatoprotective action of same extract in the present study. *In vivo* induction of CCl_4 stimulates severe oxidative stress by producing two trichloromethyl radicals in the presence of hepatic mixed function oxidase (Cyto P_{450}), which reported to induce lipid peroxidation, alteration in cell membrane permeability and mitochondrial function, thereby producing reactive oxygen species (ROS) and creating tissue inflammation and necrosis (Thanh et al., 2015). ESEt displayed radical scavenging activity in both test groups treated with same extract (600 and 800 mg/kg) by showing decrease in percent inhibition of CAT, SOD, reduced GSH and increase in percent inhibition of LPO whereas an entirely opposite picture was observed in CCl_4-induced hepatotoxic group for these four parameters. The antioxidant potential of *C. anthelminticum* seeds might be residing in their polyphenolic content, especially flavonoids. Different extracts of *C. anthelminticum* seeds proved the presence of polyphenols and flavonoids having radical scavenging abilities in *in-vitro* assay (Ani and Naidu, 2011; Mudassir and Qureshi, 2015; Shah et al., 2007).

Conclusion

The results concluded that ethanolic seeds extract of *C. anthelminticum* has potent activity to reverse the harmful effects of carbon tetrachloride on liver tissues. However, further work on the same theme has to be done on its isolated compounds to know which would be the active principle in same extract having hepatoprotective activity.

Conflict of interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Fixed and volatile constituents of *Croton heliotropiifolius* Kunth from Bahia-Brazil

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Essential oils from *Croton heliotropiifolius* Kunth roots and stems, popularly known in Bahia (Brazil) as velame or cassutinga, were obtained by hydro distillation. Fixed oil from the roots of this species was obtained by maceration in hexane. The oils were analyzed by nuclear magnetic resonance (NMR) and gas chromatography coupled with mass spectrometry (GCMS). 46 compounds were identified in the stems and 35 substances were found in the roots. The principal components oils were camphor, β-pinene, and α-pinene in the stems, and camphor, borneol, valencene, and viridiflorol in the roots. The fixed oil contained C8 to C18 fatty acids. It is the first study that reports the presence of fixed oil from the roots of this genus.

Key words: *Croton heliotropiifolius*, Euphorbiaceae, essential oils composition, fixed oil composition, velame.

INTRODUCTION

Croton is distinguished by its morphological diversity and interintraspecific and it is the second largest genus of Euphorbiaceae, comprising around 1,200 species distributed in tropical and subtropical regions, particularly in America. Brazil is a country that congregates a large number of species, about 350 species (Lima and Pirani, 2008).

Several *Croton* species have been used in traditional medicine for different purposes (Randau et al., 2004; Vunda et al., 2012). The genus contains various substances with biological activities such as against cancer – diterpenoids (clerodane, furoclerodane and acyclic diterpenes), alkaloids (taspine), and essential oils (Salatino et al., 2007). Essential oils have also been evaluated for their larvicidal efficacy against *Aedes aegypti*, amoebicidal, antifungal, and antimicrobial activities (Salatino et al., 2007; Souza et al., 2006; Rodrigues et al., 2009; Motta et al., 2013). These oils present variable components depending on the climate and soil conditions in which the specimens are found (Maia and Ming 1998).

Amongst the species of *Croton* is found *Croton heliotropiifolius* Kunth (synonymy of *Croton rhamnifolius var. heliotropiifolius*), popularly known in Bahia (Brazil) as

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velame or cassutinga, is an aromatic plant, endemic species in the Northeast of Brazil, used in folk medicine as tea for gastrointestinal diseases and to alleviate fever (Randau et al., 2004). Depending on its location, *C. heliotropiifolius* shows great variation in size and shape of the leaves, fuzz color and length of inflorescences. It is a shrubby species that ranges from 0.7 to 2.5 m in height; it may have colorless latex or orange when oxidized. The branches are cylindrical and gray-green. Alternating leaves sub-opposite the apex of the branches and may be the entire or serrated margin. It can be distinguished from other species mainly by tripartite columella of fruit at the apex after dehiscence of the fruit (Silva et al, 2010).

Some previous studies presented variations in the composition of oils from *C. heliotropiifolius* stems due to the collection period and the region where the specimen was collected (Araújo-Neves and Camara, 2012; Souza et al. 2010). Araújo-Neves and Camara (2012) identified the volatile constituents of this species present in the stems collected in the region of Pernambuco, Brazil, identified guaiol (18.38%), β-elemene (17.28%) and valerianol (10.62%) as significant components of the oil. Souza et al. (2010) identified as major components of oil from stems as the α-pinene, β-pinene, camphor and germacrene D and found a significant seasonal variation in major constituents, especially the decrease in concentration in the dry season (winter).

Considering the above, this study aimed to analyze the chemical composition of volatile and fixed oils extracted from the stems and roots of *C. heliotropiifolius* Kunth and to check the alterations of the constituents according to region where the specimen was collected. Moreover, this is the first phytochemical investigation of constituents of fixed and volatile oils presents in the roots of *Croton*.

MATERIALS AND METHODS

Plant material

Roots and stems were collected in the Morro do Chapéu City located at 1011 m above sea level with highland tropical climate in the Chapada Diamantina, Bahia, Brazil. The *Croton heliotropiifolius* was identified and a voucher specimen is deposited in the Alexandre Leal Costa Herbarium of the Institute of Biology, at the Federal University of Bahia, Brazil under number 106168.

Oil isolation

Around 100 g of material was triturated and then subjected to hydro distillation for three hours in a modified Clevenger-type apparatus. The oil was collected from a condenser, dried over anhydrous sodium sulfate, and stored at 4°C till it was analyzed. The other portion of dried roots (784 g) of *C. heliotropiifolius* was macerated with 500 mL of *n*-hexane at room temperature. The oil was filtered and 6 mL were obtained. The fixed oil was purified by column chromatography in silica gel eluted with 20 mL of *n*-hexane and dichloromethane (8:2).

Chemical analysis of the oils

The oils were analyzed by gas chromatography coupled with mass spectrometry (GC-MS), the analyses were performed on a Shimadzu QP 2010 model mass spectrophotometer with ionization source of 70 eV and with a split/splitless injector, and a Shimadzu AOC-20i auto injector (Shimadzu, Kyoto, Japan). The volume of 1.0 µL of pure oil for each sample was injected at 220°C in a DBWAX column (polyethylene glycol) 30 m × 0.25 mm × 0.25 µm film thickness (Agilent J&W DB-WAX, Santa Clara, California). The analysis occurred with 1-minute sample time in the split mode, a column flow of 1.3 mL/min, a linear velocity of 41.4 cm/s, and scan between *m/z* 40 and 500. The oven temperature was initially set at 50°C. It was increased by 20°C/min until 240°C was reached, maintaining this temperature for 5 min. The temperature was then once again increased this time by 5°C/min until a temperature of 280°C was reached, maintaining this temperature for 30 min. Inlet pressure: 37.1 kPa. Carrier gas: He, linear velocity ($\bar{u}$): 32.4cm/sec. Injection mode: split (10:1). For identification of compounds, the peaks were compared with some standards, always consulting the libraries WILEY version nº. 7 and NIST version nºs. 12 and 62.

Identification of constituents of oils

The chromatograms are shown in Figure 1 and their compositions are presented in Tables 1 to 3. The constituent's fragmentation patterns in the mass spectra were compared with those from the libraries WILEY version nº 7 and NIST version nº 12 and 62.

RESULTS AND DISCUSSION

Essential oil composition

Sesquiterpenes were the major components in all the root and stem oils studied. These results suggest that regarding the distribution and accumulation of monoterpenes and sesquiterpenes of the root and stem oil from *C. heliotropiifolius*, the chemical composition of the oils is similar to that obtained by Araújo-Neves and Camara (2012) and Souza et al. (2010).

The fatty acids present in the fixed oil were oleic, stearic, pelargonic, and palmitic acids. This was first observed in the family of the latter substance. Prior to this research, fixed oil was only obtained from the stems of *C. cajucara* Benth. (Souza et al., 2006).

Chemotaxonomic analysis

The variability in the composition of oils shows a characteristic chemical profile of the genus *Croton*.

In the stems, the main components found were the monoterpenes camphor, β-pinene, and α-pinene respectively. The α-pinene is a terpene with anti-inflammatory, antifungal, and antioxidant activity and was seen in others species of *Croton*. β–pinene, which has antifungal efficacy, was found in a smaller number of species such as *C. argyrophylloides, C. zenhtneri*, and *C. nepetaefolius* (Morais et al., 2006).

Table 1. Major constituents in the essential oil stems (OS) from *C. heliotropiifolius*

Compound	RT	Area %	IRlit[15]	I.R exp
α-thujene	5.67	0.17	924	927
α-pinene	5.90	7.21	932	933
camphene	6.37	2.27	946	943
β-pinene	7.18	7.65	974	978
myrcene	7.46	0.35	988	991
p-cymene	8.68	0.52	1020	1025
limonene	8.85	0.59	1024	1030
eucalyptol	8.972	2.26	1033	1032
linalool oxide trans	10.37	0.20	1084	1086
linalool oxide cis	10.99	0.44	1067	1069
linalool	11.52	3.54	1095	1098
octanediol	11.71	0.29	1079	1073
pinocarveol (trans)	13.23	0.72	1135	1141
camphor	13.53	17.97	1141	1149
pinocarvone	14.11	0.17	1160	1164
borneol	14.55	4.50	1165	1173
myternol	14.81	0.18	1194	1191
terpineol	14.90	1.91	1130	1137
α-terpineol	15.53	3.24	1186	1195
tridecenyne	16.02	0.27	1319	1319
thymol	19.63	0.47	1289	1293
undecanone	19.78	0.27	1293	1294
geranyl acetate	23.45	0.65	1379	1380
β-elemene	23.90	0.18	1389	1398
caryophyllene	25.14	1.20	1417	1424
azulene	25.79	0.44	1491	1490
humulene	26.62	0.29	1452	1454
germacrene D	27.68	1.03	1484	1487
eremophilene	27.95	0.59	1486	1491
valencene	28.26	2.99	1496	1492
α-amorphene	28.41	0.98	1483	1482
d-cardinene	29.20	0.22	1522	1518
germacrene B	30.77	0.87	1559	1557
nerolidol	30.95	0.72	1561	1564
ledol	31.82	2.14	1590	1593
guaiol	32.28	0.69	1600	1614
rosifoliol	32.66	0.63	1600	1609
γ-eudesmol	32.93	2.05	1630	1632
intermedeol	33.06	0.78	1665	1647
humulene epoxide II	33.40	2.14	1608	1613
spathulenol	33.47	1.11	1577	1576
hinesol	33.78	2.37	1640	1645
α-cadinol	34.07	1.19	1652	1659
β-patchoulene	34.43	2.55	1432	1379
β-eudesmol	34.48	3.90	1649	1593
elemol	35.01	1.16	1548	1522

Camphor is an antiseptic found in the species *C. crassifolius* Geisel (Yang and Deng, 2009), *C. regelianus* Muell. Arg. (Torres et al., 2008), *C. cajucara* Benth (Souza et al., 2006) and *C. hieronymi* Griseb and the

Table 2. Major constituents in the essential oil roots (OR) from *C. heliotropiifolius*

Compound	RT	Area %	IRlit[15]	I.R exp
α-Pinene	5.90	3.92	933	933
Camphene	6.36	1.73	946	943
β-Pinene	7.18	2.73	974	978
Cymene	8.68	0.29	1020	1025
Limonene	8.85	0.37	1024	1030
Eucalyptol	8.97	3.08	1033	1032
Linalool	11.51	1.80	1095	1101
Camphor	13.51	15.21	1141	1149
Camphene hydrate	13.83	0.35	1145	1156
Borneol	14.57	12.05	1165	1173
Myrtenol	14.83	0.57	1191	1191
Terpinenol	14.90	1.38	1130	1137
α-Terpineol	15.53	2.58	1186	1195
thymol	19.63	0.32	1289	1293
Methyl eugenol	24.35	0.32	1403	1361
Cyperene	24.45	0.33	1403	1407
Caryophyllene (E)	25.14	0.67	1417	1424
Azulene	25.80	1.57	1298	1290
Eremophilene	27.96	2.70	1486	1491
Valencene	28.27	9.43	1496	1492
α-Guaiene	28.42	1.31	1436	1438
Elemol	30.38	0.79	1548	1546
Nerolidol	30.94	0.51	1561	1564
Spathulenol	31.82	0.36	1577	1576
Ledol	32.16	0.45	1602	1530
Guaiol	32.28	1.02	1600	1614
Rosifoliol	32.65	0.48	1600	1609
Humulene epoxide II	32.75	0.86	1608	1630
γ-Eudesmol	32.93	1.49	1630	1632
Intermedeol	33.06	1.42	1665	1668
Globulol	33.40	1.95	1590	1530
Hinesol	33.78	1.25	1640	1645
α-Muurolol	34.18	1.91	1644	1651
Viridiflorol	34.46	8.63	1592	1594
Hexadecanoic acid	48.04	0.53	1959	1987

Table 3. Major constituents in the fixed oil roots from *C. heliotropiifolius.*

Compound	RT	Area %	IRlit[15]	I.R exp
nonanoic acid (pelargonic)	25.72	7.2	1267	1371
methyl-hexadecanoate (methyl palmitate)	44.14	14.27	1927	1925
n-hexadecanoic acid (palmitic)	45.30	13.84	1959	1977
9-octadecenoic acid (oleic)	49.65	20.87	2077	2085
octadecanoic acid (stearic)	50.51	10.04	2141	2167

borneol in *C. hieronymi* Griseb (De Heluani et al., 2005) and *C. zambesicus* (Usman et al., 2009).

In the roots, the monoterpenes camphor (antiseptic)and borneol (gastroprotective), the sesquiterpenes valencene

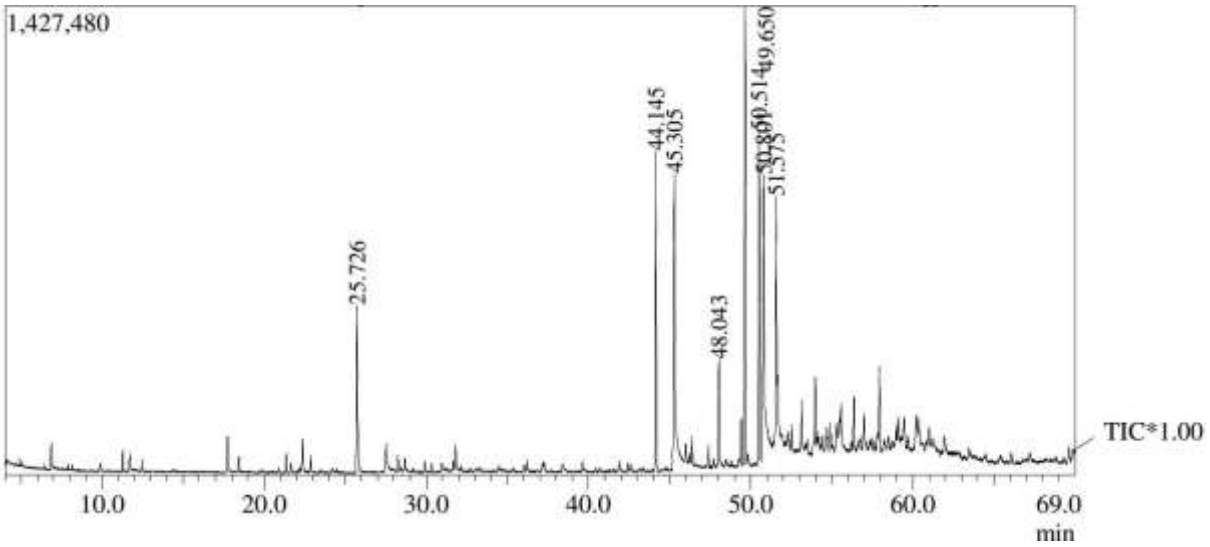

Figure 1. Cromatogram of fixed oil from roots from *C. heliotropiifolius.*

(flavor and mild oxidant) and viridiflorol (antiseptic, antibacterial, and anti-inflammatory) are the main constituents. Valencene was found in the leaves of *C. matourensis* and flowers of *C. micans* Swarts and viridiflorol has not been found in others species of *Croton* (Compagnone et al., 2010). Borneol is reported principally in barks of *Croton urucurana* (Simionatto et al., 2007).

Conclusion

This paper describes for the first time the chemical composition of the fixed oil present in C. *heliotropifolius*'s roots, as well as the volatile oils found in the roots and stems of this species with some differences from previous studies, which may be related to the region wherein the plant was collected and the stress level in the specimen used was submitted. Besides contributing to chemosystematics of the genus, this study has the perspectives to investigate the biological activities of the extracts; the phytochemical study of the polar extracts and other parts of the plant, as well as research for the future use of oil as cosmetics. It emphasizes the importance of the study of *C. heliotropifolius* found in the semi-arid of Bahia in order to contribute to the knowledge of the caatinga biome, unique in the world and has huge variety of plant species, but is threatened by human action. This knowledge can help promote their better preservation, creating jobs and reducing regional inequalities through sustainable exploitation of this and other plant species.

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Conflict of interest

Authors declare that there are no conflicts of interest.

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