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Full Length Research Paper

Phytochemical screening chemical composition and toxicity of *Euphorbia regis-jubae* (Webb & Berth)

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Plant-derived substances have recently become of great interest owing to their versatile applications. According to Ncube et al. (2008) medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. Therefore, *Euphorbia regis-jubae* (Webb & Berth), an endemic Moroccan medicinal herb, and also, widely used as food for camels and goats was subjected to a phytochemical screening of increasing polarity gradient (petroleum ether, iso-hexan, chloroform, ethanol, methanol, and distilled water). Furthermore, the chemical compositions of different solvent extracts were subjected to gas chromatography-mass spectrometry (GC-MS) analysis and the aqueous extract was purchased to an acute and chronic toxicity using Wistar rats. The acute and chronic toxicity study showed no toxicity when up to 5 g/kg body weight is administered orally. Preliminary phytochemical analysis of *E. regis-jubae* (Webb & Berth) revealed the presence of phenol, tannin, steroids, flavonoids, alkaloids, and terpenoids. Further, the GC-MS analysis revealed some compounds which are biologically important.

Key words: *Euphorbia regis-jubae*, toxicity, phytochemical screening, gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

In recent years, interest on plant research has increased all over the world. About half of all compounds that were successful in clinical trials in the last twenty-five years were, at least, have been derived from natural origin (Newman and Gragg, 2007; Qi et al, 2010; Dev, 2010).

Euphorbiaceae is among the plants known for their medicinal value and therapeutic potentials; it is one of the largest families of the Phylum Anthophyta. In this family, the largest genus is *Euphorbia*, which comprises well over 2000 species, and grows in the form of laticiferous herbs, shrubs, and small trees, inhabiting the tropical and temperate zones of Asia and other parts of the world (Heywood, 1998). Generally, they have characteristic milky latex (Mitich, 1992) with the common name "spurge weed". *Euphorbia* is stated to possess inflammatory, antiarthritic, antiamoebic, spasmolytic, antiviral, hepato-protective, and antitumor activity (Shimura et al, 1990; Semple et al, 1998; Bani, 2000; Tona et al, 2000). However, for centuries, plants and plant materials of *Euphorbia* have been known to be poisonous to human beings and often held responsible for the poisoning of livestock.

In order to find new natural sources of medicinal plants and because very little is known about the toxicity, chemical composition and efficacy of *Euphorbia regis-jubae* (Webb & Berth) belonging to the *Euphorbiaceae* family, this study aims to investigate the possible toxicity of the aqueous extract of this plant after acute and chronic administration to rats. An attempt has also been made to investigate the screening and the analysis of the

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components of several solvent extracts.

MATERIALS AND METHODS

Plant

The whole plant of *Euphorbia regis-jubae* (Webb & Berth) was collected from Sidi Ifni, Southern Anti-Atlas, Morocco and was authenticated by Professor Leila EL GHAZI of the Department of Biology, Faculty of Sciences, University of Hassan II.

To avoid any contamination or dust, the plant's aerial parts were cleaned and spread to dry at room temperature in a clean room.

Preparation of extracts

The air-dried plant material was grounded to powder form in a blender and extracted successively with iso-hexan, chloroform, ethanol, and methanol in the order of increasing polarity of solvent.

Sixty grams of the powder was extracted three times in 180 ml of iso-hexan for 15 min under agitation at room temperature. The solution is filtered on gauze through Whatman No. 1 filter paper. The filtrates were concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40°C to achieve 75 ml of solution (HI). On residual marcs we added 180 ml of chloroform for 15 min, after filtration and concentration, the chloroformic filtrate obtained was named CII. The same operation made it possible to obtain ethanolic and methanolic filtrates (EIII, MIV) (Nemlin and Brunel, 1996).

Ten grams of the powder was extracted in 100 ml of boiling water for 15 min under agitation at room temperature. The extract was filtered using gauze and Whatman No. 1 filter paper, the filtrate represents the infused solution (IFV) (Nemlin and Brunel, 1996).

Twenty grams of the powder were mixed with 200 ml of distilled water in a 500 ml round-bottom flask, linked to a column connected to a refrigerant. The round-bottom flask was placed at 60°C for 1 h. The decoction extract was filtered as mentioned earlier and concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40°C to 75 ml (DVI).

Phytochemical screening

The extracts of the plant were screened for saponosides, alkaloids, flavonoids, tannins, terpenes, sterols, phenols, saponins, and reducing compounds as described by Bekro et al. (2007), Karumi et al. (2004), Wagner (1983), Ronchetti and Russo (1971), Hegnauer (1973), Edeaga et al. (2005), and Benmahdi (2001). The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents as shown in Table 1.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of the samples of *E. regis-jubae (*Webb & Berth*)* was performed using Agilent GC-Mass 6890 model gas chromatograph-5973N model mass spectrometer equipped with a 7683 series auto-injector (Agilent, USA). The system is equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 m film thickness, Agilent, USA). The temperature was programmed from 50 to 260°C at 15°C min^{-1} and then held at 260°C for 20 min. Helium gas at a constant flow rate of 1 ml min^{-1} was used as carrier gas. The samples of 1.0 µl were manually injected in the split less mode. MS interface temperature was 230°C. For GC mass detection, an electron ionization system with ionization energy of 70 eV was used and the scan range was 30 to 700 amu.

Identification and percentage composition of the compounds was performed using MS library and the National Institute of Standards and Technology (NIST) 98 spectrometer data bank.

Acute and sub-chronic toxicity tests

The test concerned 35 male adult Wistar rats weighting between 180 to 210 g. They were housed in polypropylene cages with 12 h dark and 12 h light circle and were fed *ad libitum*.

Acute toxicity test

Acute toxicity test was performed according to the World Health Organization (WHO, 2000) guideline (World Health Organization, 2000) and the Organization of Economic Co-operation and Development (OECD, 2001) guideline for testing of chemicals 420. A group of 5 rats received a single oral dose of 5 g/kg body weight of aqueous extract, while the control group was given a saline solution (NaCl, 0.9%). The animals were observed closely for general toxicity signs, behavioural changes, mortality, and body weight for the first 6 h, then daily for 15 days.

Sub-chronic toxicity

A total of 30 male rats were randomly allotted to the control and the extract treated groups. The doses used were 0.5, 1.5, 2.5, 3.5, and 5 g/kg of the extract. The doses were administered orally daily for a period of 70 days. The control group received a solution of NaCl (0.9%). The animals were observed closely for any behavioural changes, body weight changes, and mortality and were later sacrificed for internal macroscopic, haematological, and biochemical investigations.

Biochemical parameters

Following the sacrifice, the fasting blood glucose was determined in blood plasma by glucose oxidase method (Trinder, 1969). Total cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol) and triglyceride using blood serum, were estimated by modified enzymatic method from Sigma Diagnostics (Wassan et al., 2001).

The effect of the aqueous extract on certain biochemical parameters were also examined and compared with those of the control group. The blood samples collected with heparinized tubes were centrifuged at 5000 rpm for 10 min to obtain clear plasma for the following tests: urea was determined according to Urease-Berthelot method (Weatherburn, 1967) and plasma creatinine was estimated using Jaffe reaction (Perone et al., 1992). Alanine amino transferase (ALT) and aspartate amino transferase (AST) also known as glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), respectively were measured by using enzymatic method (Horder and Sampson, 1991).

The samples were analyzed for haemoglobin (Hb) content by cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984).

Statistical analysis

All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's t-test. $P < 0.05$ was considered significant.

Table 1. Reagents and tests of chemical group's characterization.

Chemical group	Reagent	Reaction indicating that the test is positive
Flavonoids	Cyanidine	Heat then pink-orange or purplish colouring
Polyphenols	Ferric chloride	Blackish-blue or green ± dark colouring
Alkaloids	Dragendorff	Precipitate or orange colouring
	Buchard	Reddish-brown precipitate
Catechic tannins	Stiasny	Precipitate in large flakes
Gallic tannins	Stiasny	blue-black deep colouring
Polyterpenes and sterols	Liebermann	Crimson or purple ring, changing blue then green
Heterosides		Fugacious purple colouring
Reducing compounds	Keller-Kiliani	2 phases : reddish-brown colouring (acetic acid) and blue or green colouring (sulfuric acid)
Heterosidic triterpene	Salkowski	Reddish-brown colouring of the interface

Table 2. Phytochemical analysis of *E. regis-jubae* (Webb & Berth).

Extract/Compound	Iso-hexane	Chloroform	Ethanol	Methanol	Aqueous (infused)	Aqueous (decoction)
Flavonoids	-	+	+	+	+	+
Polyphenols	+	+	+	+	+	+
Alkaloids	- (B/D)	+ (B/D)	- (B) + (D)	+ (B/D)	+ (B/D)	+ (B/D)
Catechic tannins	+	+	+	+	+	+
Gallic tannins	-	+	+	+	+	+
Polyterpenes and sterols	-	+	+	+	+	+
Heterosides	+	+	+	+	-	-
Reducing compounds	+	+	+	+	+	+
Heterosidic triterpene	+	+	+	-	-	-
Saponins	-	-	-	-	-	- (Foam = 0.8 cm)

(+): Presence, (-): Absence, B: Buchard, D: Dragendorff.

RESULTS AND DISCUSSION

The results of phytochemical analysis are listed in Table 2, all the solvent extracts revealed the presence of phenolic compounds, tannins, and reducing compounds. In one hand, Iso-hexan (low polarity) was negative for flavonoids, sterols, gallic tannins, and alkaloids, while in the other hand it was positive for catechic tannins, saponosides, and polyphenols. Aqueous extracts (high polarity) do not only show the absence of saponosides (heterosides and triterpenes), but also saponins; showing foam equal to 8 cm (<1 cm).

It is reported that antioxidant effect of a plant is mainly due to phenolic compounds such as flavonoids, phenolic acids, tannins, and phenolic diterpenes (Polterait, 1997).

Phytosterols (plants sterols) are a group of naturally substances occurring in plants as bioactive minor constituents, they contribute to lowering serum cholesterol levels (Cherif et al., 2010a). Moreover, saponosides may interact with several metabolic pathways or insulin metabolism and influence directly and indirectly on glucose homeostasis (Pranav et al., 2008). This can lead to admit that the screening of *E. regis-jubae (*Webb & Berth*)* revealed the presence of different functional groups as it is reported that different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases (Liu, 2003). However, the pharmacological actions of the plant cannot be ascertained by the result of the phytochemical analysis only. The acute toxicity results showed

that all rats fed with the aqueous extract, by gastric gavage; at a dose of 5 g/kg stayed alive during the 15 days of observation. The animals did not show visible signs of acute toxicity. It suggested that the LD_{50} of the total extract was higher than 5 g/kg. However, the body weight gain of treated rats was lower than those of control group (Table 9).

After 90 days of oral chronic administration, significant gain weight was observed in both control and treated rats; but it was comparably less than groups treated with extract as shown in Table 9. Besides, treated groups showed a slight hypo-activity comparatively to the control.

In the biochemical parameters (Table 10), no statistically significant differences exist in hemoglobin (6.8%), creatinine (11.5 mg/L), urea (0.3g/L), and glucose (0.8 g/L). Urea and creatinine are non-protein nitrogenous substances are said to be end products of protein metabolism that must be removed continually (Guyton, 1981). The insignificant changes in creatinine and urea levels suggested that the activity of protein metabolism was maintained within the normal range due to relatively non-toxic effect of the extract. Triglycerides exhibited fluctuation, while SGPT and SGOT decrease with dose; these two classical enzymes are reliable indices of liver toxicity (Hayes, 1989). So, this may implied that the extract has a hepato-protective effect. A slight decrease in blood total cholesterol, HDL and low density lipoprotein (LDL) was noted at 5 g/kg which demonstrate the presence of hypolipidemic agents. Generally, all parameters were within the normal.

The results obtained from acute toxicity showed that the aqueous extract of *E. regis-jubae* (Webb & Berth) demonstrated high safety margin, since the animals tolerated up to 5 g/kg. In toxicity rating by joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives (WHO, 1966), if at 2 g/kg oral dose no death occurred, it is sufficient to assume the substance to be non toxic. In chronic study, when the extract was administered daily to the animals for a period of 60 days, no mortality or morbidity was observed. Besides in chemical tests, the significant changes are within normal ranges and showed a slight decrease in cholesterol, HDL, LDL, and liver enzymes leading to the presence of hypolipidemic agent which is an indication of the extract potential beneficial effect against cardiovascular risk factor (Zhou et al., 2006).

The results of the GC-MS analysis identified the various compounds present in the plant. Iso-hexan (Table 3) extract showed many sesquiterpenes such as: α-bulnesene, germacrene D, ledol, γ-cadinene, and β-maaliene. Sesquiterpenes are a class of terpenes that consist of three isoprene units and have the mole-cular formula $C_{15}H_{24}$. Like monoterpenes, sesquiterpenes could be acyclic or contain rings, including many unique combinations. Some kinds of sesquiterpene compounds induced apoptosis in cancer cells (Furuya et al., 1994; Woynarowski et al., 1997), and costunolide also exhibited preventive effects on intestinal carcinogenesis (Mori et al., 1994). Chloroformic extract showed the presence of pheromones such as nonacosane, triacontane (Table 4), While both infused and decoction extracts revealed the presence of glibornuride (Table 7 and 8) known as anti-diabetic drug from the group of sulfonylureas (Haupt et al., 1971) and also the presence of analgesic compound; a study reported that this synthetic diester of morphine has a duration of analgesia which is intermediate between the durations of morphine (M) and diacetylmorphine (DAM). Thus, in rats *in vivo*, 3, 6-dibutanoylmorphine (DBM) is an effective analgesic and has a reasonable duration of action release to other opioids (Tasker and Nakatsu, 1984). Both Both ethanol and methanol extracts (Table 5 and 6) showed the presence of morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5α,6α)-, vitamin E (α, β, δ-toxhopherol), and β-sitosterol a phytosterol that reduces blood levels of cholesterol, and is sometimes used in treating hypercholesterolemia. β-Sitosterol inhibits cholesterol absorption in the intestine (Matsuoka et al., 2008). When the sterol is absorbed in the intestine, it is transported by lipoproteins and incorporated into the cellular membrane (Awad and Fink, 2000). Phytosterols and phytostanols both inhibit the uptake of dietary and biliary cholesterol, decreasing the levels of LDL and serum total cholesterol. Because the structure of β-sitosterol is very similar to cholesterol, it takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen (Moreau et al., 2002). This causes less cholesterol absorption in the body. Ethanolic extract also revealed an antimicrobial compound called Solavetivone (Table 5), a sesquiterpene phytoalexins members of this family of compounds are known to inhibit mycelial growth of Phytophthora infestans (Engstrom et al., 1999) and Rhizoctonia solani (Yao et al., 2003), spore germination of *Fusarium oxysporum* (Yokose et al., 2004), and the growth of the bacteria *Staphylococcus aureus* and *Bacillus subtilis* (Kuroyanagi et al., 1998). Finally, the decoction identified a large variety of compounds which are biologically important stating with antioxidants such as: butylated hydroxytoluene (BHT) , vitamin E and linoleic acid (Table 8); however, experiments on linoleic acid subjected to 2,2′-azobis (2-amidinopropane) dihydrochloride-induced oxidation with different combinations of phenolics show that binary mixtures can lead to either a synergetic antioxidant effect or to an antagonistic effect (Peyrat-Maillard et al., 2003). It also revealed antimicrobial compounds such as lauric acid; a saturated fat which exhibit anti-microbial activity and help combat pathogenic or disease-causing viruses, bacteria and other micro-organisms (Hornung et al., 1994; Kristmundsdottir et al., 1999; Sadeghi et al., 1999). This extract also showed the presence of Lupeol which displayed antiprotozoal, antimicrobial, antiinflammatory, antitumor, and chemopreventive properties (Margareth et

Table 3. Chemical compositions of hexanic extract of *E. regis-jubae* (Webb & Berth).

Component	RT (min)	Area (%)
α-Pinene	2.38	0.07
Limonene	3.09	0.07
Nonanal	3.72	0.03
Styramate	5.05	0.06
δ –Selinene	5.71	0.02
δ-Elemene	5.74	0.02
Longifolene-(V4)	5.80	0.02
Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl- (α-Longipinene)	6.05	0.02
1H-cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1ar-(1aα,3aα,7bα)]- (β-Maaliene)	6.08	0.02
3H-3a,7-methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3ar-(3aα,4β,7α)]- (Cyperene)	6.23	0.02
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1s-(1α,7α,8aβ)]- δ-Guaiene (α-bulnesene)	6.28	0.03
α-Caryophyllene	6.61	0.04
Borneol (Camphor)	6.67	0.04
γ-Selinene	6.76	0.09
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(e,e)]- (germacrene D)	6.80	0.15
Ledol	6.97	0.01
γ-Cadinene	7.07	0.09
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (Germacrene D-4-ol)	7.42	0.07
Dodecanoic acid, ethyl ester (Lauric acid)	7.49	0.08
Dibutanoylmorphine	7.96	0.8
Oxirane, tetradecyl	8.20	0.15
Tridecanoic acid, 12-methyl-, methyl ester (methylisomyristate)	8.25	0.12
Tetradecanoic acid, ethyl ester (Myristic acid)	8.63	0.15
Methocarbamol (Miolaxene)	8.69	0.06
β-Elemene	9.54	0.58
Thunbergen	9.58	0.65
Hexadecanoic acid (Palmitic acid)	9.64	0.83
1H-cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aβ,7α,7aβ,7bα)]- (Alloaromadendrene)	9.81	0.07
1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [s-(e,z,e,e)]- (cembrene)	9.95	0.33
Phytol	10.20	0.57
Octadecanoic acid, ethyl ester (Stearic acid, ethyl ester)	10.55	0.52
Linolenic acid ethyl ester	10.46	0.99
Thunbergol	12.11	0.82
Alpha tocopherol	16.53	0.39
Taraxasterol (beta sitosterol)	21.07	0.09
Lup-20(29)-en-3-ol, acetate, (3β)-	23.86	0.11
Hop-22(29)-en-3beta-ol	25.25	0.03

Table 4. Chemical compositions of chloroformic extract of *E. regis-jubae* (Webb & Berth).

Component	RT (min)	Area (%)
Phenol, 2,4-bis(1,1-dimethylethyl)-	7.04	0.02
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	9.06	0.05
Phytol	10.2	0.17
Oxirane, hexadecyl-	13.56	0.3
Nonacosane	14.04	2.57
Triacontane	14.88	0.42
13-Tetradecen-1 ol acetate	15.3	0.15
9,19-Cyclolanost-7-en-3-ol	19.21	0.44
9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	21.5	0.6

Table 5. Chemical compositions of ethanolic extract of *E. regis-jubae* (Webb & Berth).

Component	RT (min)	Area (%)
Pyritinol	2.69	0.25
Glycerin	4.33	2.38
Pyrrolidine	5.22	0.55
4-methoxy-4-vinylphenol	5.67	0.27
Benzophenone	7.8	0.29
bacchotricuneatin c	8.19	0.3
Linoleic acid	7.72	0.33
Solavetivone	8.5	0.15
Tetradecanoic acid (Myristic acid)	8.57	0.31
Heptadecanoic acid (margaric acid)	9.94	0.24
Phytol	10.24	0.73
Octadecanoic acid (Stearic acid)	10.48	0.76
Morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5α,6α)-	11	1.19
Bis(2-ethylhexyl) phthalate	12.08	0.16
δ Tocopherol	14.52	0.01
β Tochopherol	15.54	0.03
α Tocopherol	16.51	0.25
γ-sitosterol	19.11	0.34
9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	21.72	0.09

al., 2009).

In addition to these compounds, we not only find Glibornuride as hypoglycemic (Haupt et al., 1971), but also beta-Amyrin a pentacyclic triterpenoid which according to a study has similar properties in some respects to mianserin and might possess a sedative action (Subarnas et al., 1993), benzoylecgonine and n-hexadecanoic acid which successively exhibited analgesic and cholesterolaemic properties.

In conclusion, the present study clearly revealed the presence of several biologically active phytochemicals such us flavonoids, tannins, alkaloids, steroids, phenols, and terpenes; which could serve as potential source of drugs in herbal medicine (Capasso et al., 1997).

The toxicity study which is essential for an adaptation of the traditional medicine was conducted to identify the tolerance limits of the plant extract such as veterinarians prepared by traditional healers. According to acute and chronic toxicity results, *E. regis-jubae* (Webb & Berth) aqueous extract seemed to be non-toxic to the experimental model and showed no danger to any organs as liver and kidney according to blood parameters. However, a chronic toxicity study should be further carried out to assess the long-term safety of the extract at the different doses.

The GC mass analysis revealed that this plant is a precious source of functional agents for human health such antioxidant, hypolipidemic, hypoglycemic, anti- bacterial,

Table 6. Chemical compositions of methanolic extract of *E. regis-jubae* (Webb & Berth).

Component	RT (min)	Area (%)
Butyrolactone	2.75	0.25
Dibenzazepine (iminostilbene)	2.87	0.06
4 (H) Pyridine, N-acetyl-	3.29	0.06
Benzenamine, 2- (1 methylethyl)	4.94	0.2
2-Methoxy-4-vinylphenol	5.6	0.24
Phenol, 2,6-dimethoxy	5.9	0.22
Benzophenone	7.74	0.17
p-Menth-8(10)-en-ol, cis	7.8	0.06
(1R,3R,4R,5R) Quinic acid	8.21	1.2
Sorbitol	8.44	0.12
Kaur-16-ene	9.1	0.06
n-Hexadecanoic acid (Palmitic acid)	9.52	0.9
Phytol	10.19	0.34
Myo-Inisitol	10.42	0.28
Thunbergol	10.83	0.07
Morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5α,6α)-	10.95	0.67
Bis(2-ethylhexyl) phthalate	12.03	0.09
δ-Tocopherol	14.43	0.02
β-Tochopherol	15.43	0.01
D, α tocopherol	16.37	0.15
γ sitosterol	18.9	0.19
β Sitosterol	19.1	0.02
Lanost-8-en-3-one	19.55	0.07

Table 7. Chemical compositions of aqueous extract (infused) of *E. regis-jubae* (Webb & Berth).

Component	RT (min)	Area (%)
Methoxy-phenyl-oxime	2.83	2.63
Dibutanoylmorphine	8.02	0.41
Glibornuride	10.72	0.15
n-Hexatriacontane	16.11	0.04

Table 8. Chemical compositions of aqueous extract (decoction) of *E. regis-jubae* (Webb & Berth).

Component	RT (min)	Area (%)
Dimethylamine	1.73	1
Methoxy-phenyl-oxime	3.3	2.13
Butylated hydroxytoluene (BHT)	7.45	0.21
Dodecanoic acid (lauric acid)	7.83	1.26
Tetradecanoic acid (Myristic acid)	8.94	0.87
Isopulegol	9.03	0.08
Benzoylecgonine	9.14	0.36
n-Hexadecanoic acid (Palmitic acid)	9.94	1.31
Phytol	10.63	0.28
Glibornuride	10.72	0.05
Linoleic acid	10.79	0.4
Mono olein	11.18	0.23

Table 8. Contd.

Cedran-diol, 8s,14-	11.24	0.19
9-Hexacosene	12.75	0.47
Octadecanoic acid (Stearic acid)	12.95	1.12
Nonacosane	17.47	16.13
vitamin E (α-Tocopherol)	17.85	0.18
Beta amyrin	21.48	0.57
Lup-20(29)-en-3-one	22.06	0.64
Lupeol	23.7	0.24

Table 9. Weight gain: *E. regis-jubae* (Webb & Berth) acute and sub-chronic toxicity.

Group	Weight gain (g)
Acute toxicity	
Control	87.62
5 g/kg	72.12
Sub-chronic toxicity	
Control	225.71
0.5 g/kg	233.64
1.5 g/kg	214
2.5 g/kg	209.96
3.5 g/kg	187.38
5 g/kg	131.18

Table 10. Clinical blood chemistry of male rats in the subchronic toxicity test of *E. regis-jubae* (Webb & Berth) aqueous extract.

Group/Test	T	0.5 (g/kg)	1.5 (g/kg)	2.5 (g/kg)	3.5(g/kg)	5 (g/kg)
Hemoglobin (%)	6.7 ± 0.1	6.7	6.8*	6.83 ± 0.057	6.73 ± 0.057	6.73 ± 0.057
Urea (g/L)	0.33 ± 0.014	0.32 ± 0.042	0.34 ± 0.11	0.29* ± 0.035	0.295* ± 0.007	0.29* ± 0.028
Creatinine (mg/L)	11.91 ± 1.25	11.25 ± 0.49	11.64 ± 2.3	12 ± 1.41	11.02 ± 1.52	11.2 ± 1.69
Glucose (g/L)	0.82 ± 0.09	0.73 ± 0.07	0.88 ± 0.02	0.95 ± 0.05	0.78 ± 0.02	0.86 ± 0.05
SGOT (UI/L)	103.33 ± 15.27	93.66* ± 7.76	109.66 ± 9.5	84.33* ± 3.05	65.66* ± 6.65	50.33* ± 4.5
SGP (UI/L)	94.33 ± 4.04	84* ± 4.58	78* ± 2.64	89±1	90.33 ± 2.3	56.5* ± 6.36
Cholesterol (g/L)	0.36 ± 0.09	0.35 ± 0.005	0.38 ± 0.007	0.35 ± 0.02	0.31 ± 0.007	0.21 ± 0.007
HDL (g/L)	0.11 ± 0.007	0.10 ± 0.005	0.093 ± 0.011	0.086*± 0.005	0.09 ± 0.02	0.07* ± 0.014
LDL (g/L)	0.14 ± 0.05	0.085* ± 0.03	0.066* ± 0.02	0.085*± 0.007	0.078* ± 0.007	0.04* ± 0.01
Triglicerides (g/L)	0.48 ± 0.014	0.5 ± 0.014	1.26 ± 0.03	0.35* ± 0.05	0.59*± 0.04	0.43 ± 0.021
HDL/LDL	0.80 ± 0.12	1.21* ± 0.16	1.4* ± 0.55	1.01 ± 0.81	1.14* ± 2.6	1.75* ± 1.41

*Significantly different from control at P < 0.05. The number of rats used in each group was 5.

anti-inflammatory, and analgesic compounds.

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