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PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF ARTEMISIA ABSINTHIUM FLOWERS

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ABSTRACT

Artemisia absinthium Linn. (Wormwood) is an important perennial shrubby plant that belongs to the family Asteraceae. This study aimed to evaluate cytotoxicity of Artemisia absinthium L. using different extraction methods and solvents and To determine the extraction method and solvent of the highest extraction capacity for the cytotoxic components of A. absinthium among the methods and solvents used. In this study Artemisia absinthium flowers was extracted by two methods, maceration and exhaustively by soxhlet using petroleum ether, chloroform, acetone and ethanol 70%. All extracts were subjected to phytochemical screening and TLC using standard methods and evaluated for cytotoxicity using brine shrimp lethality bioassay. Petroleum ether (soxhlet) extract was evaluated for antioxidant activity and analyzed using GC. Total flavonoid and tannin content of acetone (soxhlet) and ethanol 70% (maceration) extracts was calculated.

Phytochemical screening and thin layer chromatographic analysis of Artemisia absinthium flowers was exhibited the presence of essential oils, saponins, tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides and coumarins. Total flavonoid content of acetone (soxhlet) and ethanol (maceration) extracts was 0.86 and 0.02 mg quercetin equivalent/mg of dry extracts respectively, both exhibited total tannin of 1.0 and 1.25 mg tannic acid equivalent/mg of dry extracts respectively. About 18 compounds were detected by GC analysis of soxhlet ether extract. Soxhlet ether extract was shown to has low antioxidant

activity (RSA, 46%) and has the highest cytotoxicity among tested extracts (LC50= 63.0 µg/ml, highly toxic) and thus it is the best to retrived cytotoxic components of Artemisia absinthium through exhaustive soxhlet extraction by petroleum ether. Soxhlet extracts revealed the highest cytotoicity and the lowest yield% in comparison with maceration extracts.

KEYWORDS: Artemisia absinthium, Antioxidant, Cytotoxicity, Thin layer chromatography, Soxhet, GC.

INTRODUCTION

For thousands of year's natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases (Phillipson 2001). According to recent studies conducted by the WHO, about 80% of the world's population relies on traditional medicine. About 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources. Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics. Between the years 1981-2006, about a hundred anticancer agents have been developed, of which, twenty five are natural product derivatives, eighteen are natural product mimics, eleven candidates are derived from a natural product, and nine are pure natural products.[1]Antioxidants are important species which possess the ability to protect the organism from damage caused by free radical-induced oxidative stress. Oxidative damage to protein, DNA and lipid is associated with chronic degenerative diseases including cataracts, cancers and coronary heart disease. However, concern about the safety of the commonly used synthetic antioxidants such as butylated hydroxytoluene had led to increasing interest in naturally occurring alternatives which occur in plants as secondary metabolites. There is currently much interest in the antioxidant role of flavonoids and other polyphenols.[2] Now days, cancer is the biggest challenge in the medical field, no ideal anticancer therapy with low side effect and high efficay along with low cost, therefor researches intensified in this field to derived anticancer medicines of natural sources with best outcome.

Wormwood (Artemisia absinthium L.) is an aromatic bitter herb, which has been used as a medicine from ancient times. It has traditionally been used as anthelmintic, choleretic, antiseptic, balsamic, depurative, digestive, diuretic, emmenagogue and in treating leukaemia and sclerosis. Extracts of wormwood have been used as a muscle relaxer and as a mild

sedative to treat anxieties. Wormwood is the aromatic spice, widely employed as a flavouring agent in wine and other alcoholic beverages. Also, to a lesser extent it is used in soft drinks and some foods. Thujone has achieved notoriety as the neurotoxic agent in worm wood oil from Artemisia absinthium (Compositae/Asteraceae), used in preparation of the drink absinthe, now banned in most countries. The useful parts are leaves, flowering tops, roots, stem and mostly all parts of the plant. They are used in chronic fevers, swellings, inflammation of liver, menstrual disorders. It is a remedy for enfeebled digestions and debility. Flowers are vermifuge, tonic in intermittent fever. Herb yields an essential oil, called wormwood oil which is used externally in rheumatism. A.absinthium has numerous flower heads with short stems and hangs in many flower panicles. Flowers are heterogamous in nature. Each flower head is surrounded by 8-10 bracts. Size of single flower is like approximately 3–4 mm (0.12–0.16 in). Capitula flowers are pale yellow and tubular.The number of stamens in the flower are 5 and its pistil is made up of 2 fused carpels. The shape of the involucre is hemispherical; they (involucral bracts) are arranged in several rows and hairy.[3]

MATERIALS AND METHODOLOGY

Collection and preparation of the plant materials

Artemisia absinthium flowers were obtained from herbalist in Omdurman market during March, cleaned from dust and foreign matter and authenticated by the Medical and Aromatic Plant Research Institute, Khartoum, Sudan on 24\3\2017.

Extraction and preparation of extracts

Artemisia absnthium flowers were crushed and extracted by maceration and exhaustively by soxhlet apparatus, each time, the extract was filtered and air dried, yield percentage of different extracts was calculated using equation: yield percentage = (weight of extract/total weight of sample)*100. The different extracts were preserved in refrigerator for further study.

Exhaustive extraction by soxhlet apparatus

50g of crushed plant material was extracted using soxhlet apparatus successively with different organic solvents in order of increasing polarity, Petroleum ether, Choloroform, Acetone and Ethanol 70%.

Maceration

Four weights of plant material (50 gm for each) were extracted by maceration for 24 hour using Petroleum ether, Choloroform, Acetone and Ethanol 70% respectively.

Phytochemical Screening of A. absinthium Flowers

General phytochemical screening for the presence of Anthraquinones, Flavonoids, Saponins, Tannins, Alkaloids and Cardiac glycosides was done using standard method with some modification.[4]

Test for Anthraquinones: 200 mg of each extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added and observed.

Test for flavonoids: Two methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an 200 mg of each extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappear on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Test for Saponins: 200 mg of each extract was dissolved in 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Tannins: 200 mg of each extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Alkaloids: 200 mg of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into three portions. Mayer's reagent was added to one portion and Hager's and wagner's reagents to the other portions. formation of a cream (with Mayer's reagent) was positive for the presence of alkaloids.

Test for Cardiac glycosides (Keller-Killiani test): To 200 mg of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Thin layer chromatographic analysis:

From each extract, about 20-100 micro were applied using capillary tubes to a precoated silica gel plates, 7 plates were prepared to cover each of the main classes of constituents. Each having different solvents system and detection method (Table 1).[5]

Table 1: Solvent systems and detection methods used in TLC of A.absinthium flowers

Plant constituent	Solvent system	Detection method
Essential oils	Toluene-ethyl acetate(93:7)	Vanillin/H_2SO_4
Coumarins	Toluene-ethyl acetate(93:7)	10% ethanolic KOH and U.V
Anthraquinones	Ethyl acetate – methanol-water (100-13.5-10)	Borntrager and U.V
Cardiac lycosides	Ethyl acetate – methanol-water (100-13.5-10)	Conc. H_2SO_4 and U.V
Saponins	Ethyl acetate –methanol-water (100-13.5-10)	Vanillin/H_2SO_4
Alkaloids	Ethyl acetate- methanol-water(100-13.5-10)	Drangendroff reagent and U.V
Flavonoids	Ethyl acetate- methanol-water(100-13.5-10)	U.V

Total flavonoid content

The total flavonoids content was determined by adopting the method described by Shanukha et al., (2012). Aliquots of each extracts were pipette out in series of test tubes and volume was made up to 2 ml with distilled water, 0.3 ml of sodium nitrite (5%) was added to each tube and incubated for 5 min. at room temperature, 0.3 ml of aluminium chloride solution (10%) was added and incubated for 5 min, 2 ml of sodium hydroxide (1M) were added. Absorbance was measured at 415 nm against a reagent blank. A calibration curve was constructed, using quercetin (0.1-0.9 mg/ml) as standard. Total flavonoids content was expressed as mg quercetin equivalent/mg of dry extract using the equation derived from the calibration curve of the standard quercetin (concentration versus Absorbance). Total flavonoid content of the extracts (mg/ml) expressed as quercetin equivalents.[6]

Total Tannin content

The tannins content was determined by using FeCl3 and gelatin test method as described by Shivakumar et al., (2012) with some modification. About 1 ml of extract (1mg/ml) was transferred to vials, 1ml of 1% K3Fe (CN) 6 and 1 ml of 1% FeCl3 were added, and the volume was made up to 10 ml with distilled water. After 5 min absorbance was measured at 510 nm against a reagent blank. A calibration curve was constructed, using tannic acid (0.1-0.8 mg/ml) as standard. The total tannins content of the extracts (mg/ml) was calculated using the equation derived from the calibration curve of the standard tannic acid (concentration versus Absorbance). Total tannin content of the extracts (mg/ml) expressed as tannic acid equivalents.[6]

GC Analysis of Petroleum ether extract

GC-MS condition: Gas Chromatography Mass Spectrometer was performed using Shimadzu (japan) instrument (GC- MS- QP- 2010 ultra). The analytical column was RTX5MS (5% phenyl- 95% dimethyl polysiloxane with length of 30 meter x 0.25 µm), the sample was injected by using split mode. Helium gas was used as a carrier gas at a flow rate of 1.61 ml/min. the temperature was programmed at 60˚C with rate of 10˚C/min to 300 as final temperature. The temperature of injector was 250˚C. The sample was analyzed by using scan mode in the range of m/z 40-550 charges to ratio and the total run time was 24 minute. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library, results were recorded.

Sample preparation (Methylation): 2 ml of the sample was transfer into a test tube, 7 ml of alcoholic NaOH (2 gm NaOH/100ml methanol) was added and the mixture was shacked for 3 min. by a vortex and left overnight then, 2 ml of supersaturated NaCl and 2 ml of normal hexane were added and the mixture was shacked for 3 min. and hexane layer was collected, 5 µl from hexane layer was diluted by 5 ml diethyl ether, 1 g of sodium sulphate was added as drying agent, the mixture was filtered by syringe filter 0.45 µm, the filtrate was transferred directly to the GC-MS vial and 1 µl was injected directly.

Antioxidant activity using DPPH method

The DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging was determined according to the method of [10] with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37° c . The concentration of DPPH was kept as (300 µM). The test samples were

dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 512 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in compassion with DMSO treated control group. All tests and analysis were run in triplicate.[7]

Evaluation of cytotoxicity by Brine Shrimp Lethality Test

The brine shrimp assay was developed by,[8] The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials.[9]

Brine shrimp lethality Bioassay is widely used as a simple, reliable and cheaper prescreens method to determine the cytotoxicity of crude plant extract and pure natural compounds, especially antitumor compounds from the natural source. The test was carried out according to the method prescribed by.[10] Eggs of Artemia salina (Family, Artemiidae) were stored at low temperatures (4ºC), they will remain viable for many years. Brine shrimp eggs, Artemia salina were hatched in artificial seawater prepared by dissolving 38g of sea salt in one liter of distilled water. After 24-72 h incubation at room temperature (37°C), the larvae were attracted to one side of the vessel with a light source and then collected with pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing artificial seawater. Brine shrimp lethality bioassay was carried out to investigate the cytoxicity of plant extracts. 50 mg of (Leach) eggs were added to a hatching chamber containing artificial Sea water (75ml). The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. 20 mg of test extracts of the various plant species were separately dissolved in 2 ml of methanol, then 500, 50, and 5µl of each solution was transferred into vials corresponding to 1000, 100, and 10 µg/ml, respectively. Each dosage was tested in triplicate. 10 larvae of A. salina Leach (taken 48–72h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5ml with Sea water immediately after adding the shrimps. One drop of DMSO was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. The numbers of survivals were counted after 24 hour, the experiment was performed in triplicate, the % of mortality was then determined and the main of mortality values was calculated. LC50 value (concentration that induce 50% mortality of brine shrimp napulii) was obtained from the best fit line by plotting conc. versus % of mortality.

Statistical analysis

Data analyzed using excel sheet 2013. Data were reported as mean ± SD.

RESULTS

Yield percentage of all extracts of Artemisia absinthium flowers

The tested extracts revealed different yields in term of %, phytochemical screening of Artemisia Absinthium exhibited the presence of some secondary metabolites (table 2).

Table 2: Yield % and Phytochemical screening of Artemisia absinthium flowers.

Extraction		Yield %	Phytochemical tested					
Solvent	Method		Anthraquinones	Flavonoids	Saponins	Taninns	Alkaloids	Cardiac glycosides
PET	Sox.	3.6	-ve	-ve	-ve	-ve	+++	-
	Mac	3.9	-ve	-ve	-ve	-ve	+++	-
$CHCL_3$	Sox.	4	+ve	-ve	-ve	+ve	+	-
	Mac	6.8	+ve	+ve	-ve	+ve	-	+ve
Act	Sox.	1.4	-ve	-ve	-ve	-ve	++	-
	Mac	2.5	+ve	+ve	-ve	+ve	-	-ve
EtOH 70%	Sox.	4.2	+ve	+ve	+ve	+ve	++	-
	Mac	8	+ve	+ve	+ve	+ve	-	-ve

Key: +ve: indicate presence of phytochemial,- ve test : indicate absence of phytochemical group, PET : Petroleum ether, $CHCL_3$: Chloroform, Act: Acetone, EtOH: Ethanol 70%, Sox: Soxhlet, Mac: Maceration.

Thin layer chromatographic analysis of all extracts of A. absinthium flowers

Chromatographic analysis of all extracts of Artemisia absinthium flowers exhibited various compounds (table 3).

Table 3: Chromatographic analysis of main classes of phytochemicals.

Extraction		Compound		Detection Method
Solvent	**Method**	**Code/R_f**	**Colour**	
Chromatographic Analysis of Essential oils in all extracts				
PET	Soxhlet	C1/ 0.19 C2/0.32 C3/ 0.90	Green (vis.) Blue (vis.) Strong blue (u.v)	Vanillin/H_2SO_4
	Maceration	-	-	
$CHCL_3$	Soxhlet	-	-	
	Maceration	C1/0.25 C2/0.29	Green (vis.) Green (vis.)	

Act	Soxhlet	C1/0.06	Green (vis.)	
	Maceration	C1/0.98	Acetone (vis.)	
EtOH 70%	Soxhlet	-	-	
	Maceration	-	-	
Chromatographic Analysis of Coumarins in all extracts				
PET	Soxhlet	C1/0.17 C2/0.24	Blue (u.v) Blue (u.v)	10% ethanolic KOH and U.V
	Maceration	-	-	
$CHCL_3$	Soxhlet	-	-	
	Maceration	C1/0.28 C2/0.5	Bluish green (u.v) Yellow (u.v)	
Act	Soxhlet	C1/0.18	Green (u.v)	
	Maceration	C1/0.18	Brown (u.v)	
EtOH 70%	Soxhlet	C1/0.27	Green (u.v)	
	Maceration	C1/0.18 C2/0.28	Green (u.v) Bluish green (u.v)	
Extraction		**Compound**		**Detection Method**
Solvent	**Method**	**Code/R_f**	**Colour**	
Chromatographic Analysis of Flavonoids in all extracts				
PET	Soxhlet	C1/0.903 C2/0.935	Brown (u.v) Green (u.v)	U.V
	Maceration	-	-	
$CHCL_3$	Soxhlet	C1/0.887 C2/0.854	Green (u.v) Green (u.v)	
	Maceration	C1/0.919 C2/0.967	Green (u.v) Gray (u.v)	
Act	Soxhlet	-	-	
	Maceration	C1/0.919 C2/0.967	Green (u.v) Orange (u.v)	
EtOH 70%	Soxhlet	C1/0.887	Green (u.v)	
	Maceration	C1/0.984 C2/0.697	Green (u.v) Orange (u.v)	
Chromatographic Analysis of Anthraquinones in all extracts				
PET	Soxhlet	C1/0.89	Yellow (vis.)	Borntrager and U.V
	Maceration	-	-	
$CHCL_3$	Soxhlet	C1/0.8	Yellow (vis.)	
	Maceration	C1/0.86	Red (vis.)	
Act	Soxhlet	-	-	
	Maceration	-	-	
EtOH 70%	Soxhlet	C1/0.76 C2/0.88	Yellow (vis.) Yellow (vis.)	
	Maceration	C1/0.77 C2/0.86	Red (vis.) Red (vis.)	
Chromatographic Analysis of Saponins in all extracts				
PET	Soxhlet	C1/1.0	Brown (vis)	$Vanillin/H_2SO_4$
	Maceration	-	-	
$CHCL_3$	Soxhlet	C1/0.975	Brown (vis)	
	Maceration	C1/0.95	Brown (vis)	
Act	Soxhlet	C1/0.925	Brown (vis)	

	Maceration	C1/0.937	Brown (vis)	
EtOH 70%	Soxhlet	C1/0.925	Brown (vis)	
	Maceration	C1/0.925	Brown (vis)	

Key: PET: Petroleum ether, $CHCL_3$: Chloroform, Act: Acetone, EtOH: Ethanol 70%, C:compound, vis.: on visible light, U.V: under ultraviolet, R_f: retention factor.

Total flavonoid and tannin content of soxhlet ethanol and acetone (maceration) extracts

Absorbance of standard Quercetin and Tannic acid was measured respectively (table 4) and accordingly the calibration curve of the standard quercetin and tannic acid respectively was set (Figure 1 and 2). Calibration curves equations used for calculation of favonoid and tannin content were found to be y = 0.8065x + 0.0349, R^2 = 0.9855 and y = 2.8072x + 0.0824, R^2 = 0.9936 respectively, where y= Absorbance of the extract, x=concentration of the standard (mg/ml) (table 5).

Table 4: Absorbance of different conc. of Quercetin and Tannic acid.

Quercetin		Tannic acid	
Conc. (mg/ml)	**A**	**Conc. (mg/ml)**	**A**
100	0.1253	100	0.3947
200	0.1937	200	0.5984
300	0.2872	300	0.9048
400	0.3337	400	1.1799
500	0.4048	500	1.6069
600	0.5382	600	1.7208
700	0.6137	700	2.0285
800	0.7234	800	2.3311
900	0.7234	-	-

Key: A: absorbance.

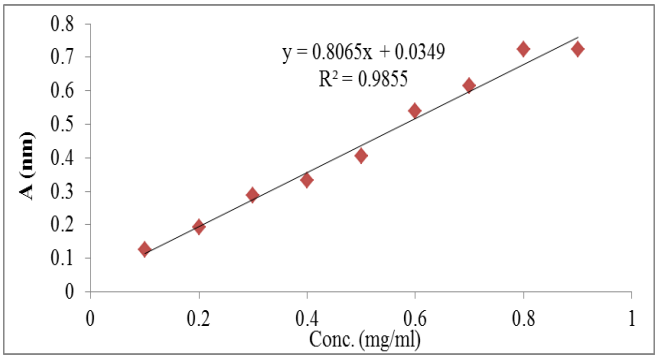

Figure 1: Absorbance of different conc. of standard Quercetin.

Key: A and Y: absorbance of the standard quercetin at ʎ 415 nm, x=conc. of quercetin (mg/ml).

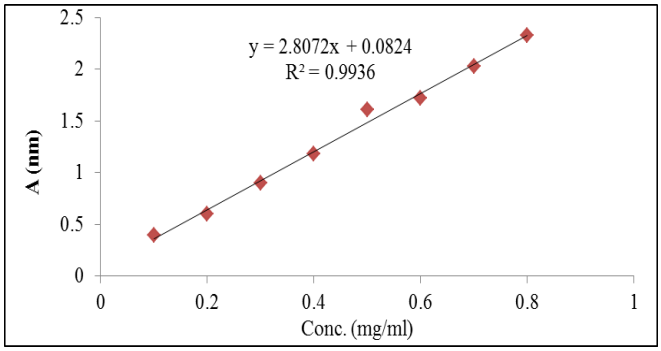

Figure 2: Absorbance of different conc. of standard Tannic acid.

Key: A: absorbance of the standard tannic acid at wave length 510 nm. y= Absorbance of tannic acid, x=concentration of tannic acid (mg/ml).

Table 5: Total flavonoid and tannin content of ethanol (maceration) and Acetone (soxhlet) extracts.

Extract	Flavenoids		Tannins	
	A(nm)	Conc.(mg QE/mg of extract)	A (nm)	Conc. (mg TAE/mg of extract)
Ethanol (Maceration)	0.0220	0.02	1.8360	1.25
Acetone (soxhlet)	0.7288	0.86	1.4863	1.00

Key: QE: Quercetin equivalent, TAE: Tannic acid equivalent.

GC analysis of petroleum ether extract

Gas chromatographic analysis of petroleum ether soxhlet extract revealed presence of about 18 compounds, most of them are fatty acids esters (table 6).

Table 6: Peak reports of GC analysis of soxhlet ether extract of A. absinthium flowers.

Peak	Compound	Area	Area%	Rt
1	1-hexanol, 2-ethyl-	1106220	5.41	4.657
2	A-Terpineol	108260	0.53	7.000
3	Decanoic Acid, methyl ester	70148	0.34	8.703
4	Dodecanoic acid, methyl ester	2028468	9.92	11.263
5	Methyl tetradecanoate	1147635	5.61	13.579
6	Pentadecanoic Acid, methyl ester	56727	0.28	14.655
7	Hexadecanoic Acid, methyl ester	5542221	27.11	15.680
8	Heptadecanoic Acid, methyl ester	73601	0.36	16.658
9	9,12-Octadecadienoic acid(Z,Z) , methyl ester	2131387	10.43	17.331
10	9-Octadecanoic acid(Z) , methyl ester	805112	3.94	17.385
11	9,12,15-Octadecatrienoic acid(Z) , methyl ester	2434739	11.91	17.401
12	Phytol	1518875	7.43	17.504
13	Methyl stearate	1596447	7.81	17.595
14	Eicosanoic acid, methyl ester	462001	2.26	19.351

15	γ-Tocopherol	192145	0.94	19.979
16	Phenol,2,2-methylenebis[6-(1,1-dimethyl ethyl)-4-methyl-	310519	1.52	20.280
17	Docosanoic acid, methyl ester	487728	2.39	20.973
18	Tetracosanoic acid, methyl ester	371193	1.82	22.475
Total		20443426	100	

Key: Rt: retention time.

Biological screening of A. absinthium flowers

Radioactive scavenging activity A. absinthium flowers

Soxhlet ether extract was shown to has low antioxidant activity (RSA, 46%) in comparison with standard antioxidant, propyl galate (RSA%, 92%).

Cytotoxicity of A. absinthium flowers

Soxhlet extracts of A. absinthium was revealed highest lethality (lowest LC50) than maceration extracts (table 7, figure 3), Toxicity profile was set according to.[11]

Table 7: Lethality of all extracts of Artemisia absinthium flower.

Extract		Conc.(µg/ml) / % Mortality				LC50 (µg/ml)	Toxicity profile	Calibration curve
Solvent	Method	0	10	100	1000			
PET	Mac.	0	63.33±9.4	70±8.2	80±8.2	195.2	Medium	y = 0.0405x +42.095 R^2 = 0.2928
	Sox.	0	70±8.2	80±8.2	83.3±9.4	63.0	Highly toxic	y = 0.0389x +47.549 R^2 = 0.2288
$CHCL_3$	Mac.	0	30±8.2	30±8.2	30±8.2	2587.5	Non toxic	y = 0.0119x +19.209 R^2 = 0.1462
	Sox.	0	60±8.2	63.33±4.7	70±8.2	327.4	Medium toxicity	y = 0.0332x + 39.13 R^2 = 0.2438
Act	Mac.	0	13.33±4.7	16.67±9.4	16.67±4.2	5128.3	Non toxic	y = 0.0079x + 9.4862 R^2 = 0.2295
	Sox.	0	26.67±4.7	30±8.2	40±8.2	1381.7	Non toxic	y = 0.0234x +17.668 R^2 = 0.44
EtOH	Mac.	0	23.33±4.7	33.33±4.7	33.33±4.2	1894.9	Non toxic	y = 0.017x + 17.787 R^2 = 0.2731
	Sox.	0	50±8.2	53.3±4.7	70±8.2	446.3	Medium toxicity	y = 0.0395x +32.372 R^2 = 0.4008

Key: PET: Petroleum ether, $CHCL_3$: Chloroform, Act: Acetone, EtOH: Ethanol 70%, Mac.: Maceration, Sox.: Soxhlet, Y: Mortality %, x: concentration of extract, SD: standard deviation, R: regression coefficient. Score of toxicity: LC50>1000 µg/ml are non-toxic, 500 - 1000 µg/ml are low toxic, 100 - 500 µg/ml are medium toxic, 0 - 100 µg/ml are highly toxic.

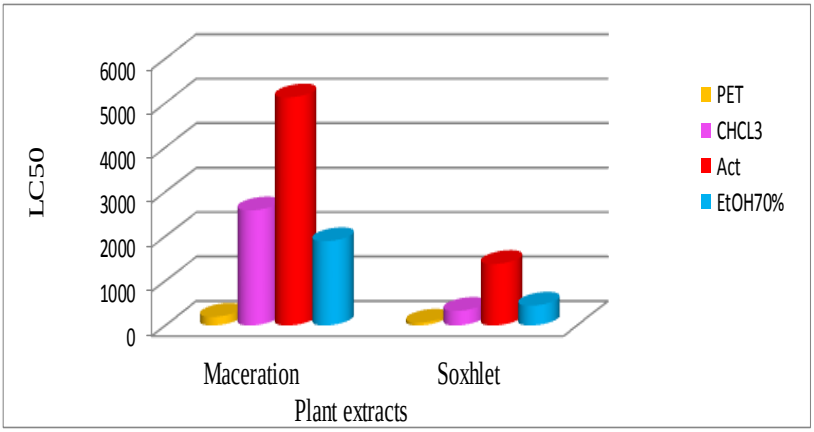

Figure 3: LC50 of all extracts of A. absinthium flowers.

Key: PET: Petroleum ether, $CHCL_3$: Chloroform, Act: Acetone, EtOH: Ethanol 70%.

DISCUSSION

The hot climate of Sudan together with various environmental factors like dust and hygene is a main causes of release of free radicals, a main cause of cancer diseases. Free radicals are implicated for more than 80 diseases including diabetes mellitus, cataract, atherosclerosis and other autoimmune disease like aging. In treatment of these diseases, antioxidant therapy has gained most importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin.[12]

There is a high incidence of cancer diseases in sudan in the recent days, chemotherapy resistance together with high cost and severe side effects make many to use alternative traditional medicines which rely mainly on the plants.

Phytochemical analysis of Artemisia absinthium revealed the presence of various metabolites that may affect the antioxidant and cytotoxicity of each other negatively or positively. Different activities was observed for the same solvent which reflect the effect of extraction method used. High tannin content of ethanol (maceration) and Act (soxhlet) extracts together with high LC50 excluded the significant participation of tannin in cytotoxicity of Artemisia absinthium, on the other hand, on carrying a comparison between flavonoid content and LC50 of ethanol maceration and acetone soxhlet extracts, the later has lowest the LC50 and

the highest flavonoid content that may reflect the role of flavonoids in cytotoxicity of Artemisia absinthium. GC analysis of ether extract (soxhlet) revealed the presence of many compounds, mostly fatty acids derivatives that may contribute in the coexisting high toxicity of Artemisia absinthium, α-Terpineol one of which.[13] conclude that Both caryophyllene and α-Terpineol showed important antiproliferative effects on K562 cells.

CONCLUSION

In the present study, from all observations and results obtained it was concluded that Artemisia absinthium revealed immense phytochemicals, tannins, flavenoids, coumarins, saponins, essential oils, alkaloids and anthraquinones with high flavonoid (observed with acetone soxhlet extract) and tannin content and exhibited high cytotoxicity that is best retrived through exhaustive soxhlet extraction by petroleum ether. Soxhlet extracts revealed the highest cytotoicity and the lowest yield% in comparison with maceration extracts.

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