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GAS CHROMATOGRAPOHY MASS SPECTROPHOTOMETRY (GC-MS) ANALYSIS OF FEMALE CAMEL URINE EXTRACTS

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المستخلص

حُلل التركيب الكيميائي لبول النوق المستخلص بواسطة الكلوروفورم، الإيثانول ومجفف بالتجميد باستخدام الغاز اللوني الطيفي الشامل (GC-MS). تم الكشف عن 17 مركب عضوي نشط حيوياً. كانت المركبات المتدهورة في جميع المستخلصات مماثلة لبعضها البعض. خلصت الدراسة إلى أن مستخلصات بول النوق يحتوي على مركبات مشطة حيوياً وهي قيمة للغاية في الكشف وتصنيع عقاقير جيدة ذات أصل طبيعي.

Abstract

In this study the chemical composition of female camel urine extracts (chloroformic, ethanolic and lyophilized) were analyzed by GC-MS: Agilent technologies' 5973N. Seventeen bioactive organic compounds were detected. The degraded compounds in all extracts were comparable to each other. The results obtained verified that female camel urine extracts are excellent poll of bioactive compounds which are extremely valuable for detection and manufacture of new drugs of natural origin.

Introduction

The use of human urine and urine extracts for medical purpose has been known for centuries (Armstrong, 1937; Burzynski, 1988). Recently the medical science of human and animal urine has a profound medical uses ,(Christy ,2000 : Peroni ,2001 :Natalie , 2002).The use of urinary remedy towards illness in Asia has been gaining high popularity(Read ,1979 :Lai et al ,1999). Use of animal urine is endorsed in mainstream modern medicine. Mare urine is the source of conjugated equine estrogens and has been marketed for over fifty years as the pharmaceutical brand premarin, "an estrogen treatment for menopausal and pre menopausal women" especially postpartum and one of the most prescribed drugs in the United States (Christy 2000). It was very recently discovered that adding distilled cow urine to medicaments increases their effectiveness while decreasing their side- effects, making anti – cancer and anti- tubercular drug twenty times more effective and anti- bacterial eighty times more effective (on line document). The reliable therapeutic efficacy obtained from clinical studies on camel urine were recorded by (Ohag, (1993, 1998); Kabariti,1988;Burziski,1977); Mona. 2003; Wisal, G. 2002; Salwa,et al , 2006). The results of these experiments proved that camel urine consists of many bioactive complex compounds which can act against bacterial, fugal, viral, parasitic and carcinogenic agents and it has the ability to protect the liver against toxic agents (Salwa, et al 2009)..Ethno pharmacology of camel urine as folk medicine and up to date for a variety of ailments is more commonly considered of therapeutic value and drug important. Significant advances have been occurred in recent years. The methods of pharmacology- pharmochemical and chromatography revealed an insights in the etiology and discovery of new drugs and compounds, however there is continuing need for vast improvement in our knowledge and understanding of discovering new compounds and drugs. The work published in this article deals with the analysis of female Camel urine and its extracts, and to investigate its bioactive chemical components.

Materials and methods

Sample collection:

Female camel urine was collected by natural urination or by tashweel technique.

Sample preparation

Lyophilized Female Camel Urine (LU):

Tow ml of Female Camel urine were poured in piqué bottles and freeze dried by Freeze Drier machine.

Chloroformic Extract :

Equal volumes of female camel's urine (FCU) and chloroform were skaking for three hours and left to separate, .the lower chloroformic layer was then displaced and analyzed by Gas Chromatography- Mass Spectrometry (GC-MS)

Ethanolic Extract

Ten grams of lyophilized female camel urine was refluxed in 80% ethanol for 30 min then filtered; the filtrate was further analyzed by GC-MS.

Analytical Methods:

Gas chromatography – Mass spectrometry (GC-MS) was performed using Agilent 6890 N Net work GC system interfaced with 5973 N Net work, Mass Selective Detector (MSD). The GC-MS was fitted with a 60 m Agilent fused capillary column, DB-5ms 0.25mm 1-D, 0.25 mm film – initial temp 100c◦, hold 2 min, then programmed at 2c◦/min to 300c◦ min, isothermal temperature was held for 10 min .

Helium carrier gas, head pressure 9.30 psi, column flow 1ml/min. injection temp. 300C◦ El source 230c◦, total scan mode was cycled at 2 seconds. I ml

of the given sample has been diluted with 10 ml of diethyl chloromethane (DCM) and 1µ was injected using split less mode .

IR Apparatus

A Perkin Elmer 2 Lambda Spectra, 580 Infra Red Spectrophotometer Neel fur was used for detecting the functional groups in LU & CE of Female Camel Urine using kBr and NaCl respectively.

Results and Discussion

Identification of the degraded compounds was obtained by comparison with published NIST Library retention time of the chromatogram .Corrected areas percentage obtained by base line subtraction were used to calculate the percentage of the compound within the injected amount. Table 1 represents the GC_MS chromatogram of Lyophilized Urine. Table 2 for ethanolic extract degraded compound; Table 3 shows the degraded compounds of chloroformic extract. Infra Red (IR) analysis of lyophilized and chloroformic extract of female camel urine was made. Table 4&5 show the obtained functional groups of (LU) and the medicinal uses of some degraded compounds respectively.

The GC-MS analysis of ethanolic extract , lyophilized and chloroformic extract of female camel urine revealed a comparable degraded compounds, these compounds contain aliphatic hydro carbon chains (3 up to 27 carbon atoms) with oxygen ,nitrogen ,silicon ,alkyl and phosphorus .Benzene rings ,phenolic ,Omiga 6 &9 compounds and some novel compounds such as titanium , oxirane and heptasiloxane were obtained . These results suggest that these chemicals may have widespread distribution in the grazing plants of camels. Some of these compounds are medically used for cancer .This was in agreement with the records of (Khorshid et al 2005). The uses of camel urine as anti bacterial , antifungal , ant parasitic , and as cosmetic ingredients were reported by Christy (2000) and Natalie(2002). Degraded compounds agreed with that in *Merck Index (1968 ; 1998 ; 2006). The presence of hydroxyl (OH), carboxylic (COOH), aromatic (C_C), amine

(NH), thiol (S=O) and chlore (CL) in female camel urine may enable camel urine and its extracts to act via different chemical pathways.

Table (1): GC-MS Degraded Compounds of Lyophilized female Camel urine

Peak	R.T.	Compounds	(b)Match	(a)%of total
1	8.13	Titanium, (Ü8-1,3,5,7-cyclooctatetraene)(Ü5-2,4-cyclopentadien-1-yl)-	749	1.33
2	8.84	4-Heptanone, 3-methyl-	904	2.30
3	10.88	Butanoic acid, butyl ester	907	2.23
4	14.87	Acetic acid, [(2,4,6-triethylbenzoyl)thio]-	807	1.90
5	15.03	Benzoic acid, methyl ester	941	8.00
6	17.76	Propane, 2,2-[methylenebis(oxy)]bis[2-methyl-	745	1.27
7	21.41	Butane, 1,1-dibutoxy-	872	26.59
8	21.67	Pentanoic acid, 4-oxo-,butyl ester	910	3.12
9	27.12	Benzoic acid, butyl ester	965	22.90
10	29.52	Benzene acetic acid, 2-methyl propyl ester	866	2.67
11	40.64	Butyl parabene	914	27.71

*(a)The lowest % reported is 0.7% - anything lower than 0.7% was omitted

*(b) The chromatogram was matched with NIST liberary-if a match is more than 800, then probably the compound is present.

Table (2): GC-MS Degraded Compounds of Ethanol extract of female Camel urine

Peak	R.T.	Compounds	(b)Match	(a)%of total
1	8.84	4-Heplanone, 3-methly-	867	3.82
2	10.89	Butanoic acid, butyl ester	851	2.41
3	14.88	Acetic acid, [(2,4,6-triethylbenzoil)thio]-	815	3.55
4	17.96	Sulfone, 2-hydroxybutyl	626	2.16
5	21.41	Butane, 1,1—dibutoxy-	858	26.17
6	55.95	Hexadecanoic acid, butyl ester	819	7.70
7	56.36	Oxirane, [(hexadecyloxy)methyl]-	899	2.49
8	59.23	Heptacosane	705	3.21
9	60.93	9-octadecenoic acid, (E)-	773	6.77
10	61.15	9-octadecenamide, (Z)-	637	3.21
11	61.64	Octadecenoic acid, 2-methylpropyl ester	734	5.23
12	62.00	Heptacosane	689	3.84
13	64.55	Heptacosane	607	3.65
14	64.92	2,4-Bis(dimethylbenzyl)-6-t-butylphenol	575	8.23
15	67.20	1,2-dihydro-2,4-diphenyl-quinazoline	593	2.98
16	71.23	Erucic acid	678	9.29
17	71.73	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl estet. (z,z,z)-	609	5.32

*(a)The lowest % reported is 0.7% - anything lower than 0.7% was omitted

*(b) The chromatogram was matched with NIST liberary-if a match is more than 800, then probably the compound is present.

Table (3): GC -MS Degraded Compounds of Chloroform extract of female Camel urine

peak	R.T	Compound	% of total
1	8.842	Heptanoic acid	1.59
2	10.642	Phathlic anhydride	1.01
3	12.908	N.decanoic acid	0.95
4	17.675	Benzoic acid	0.21
5	18.392	Cyclooctanone	0.84
6	23.750	Dodecanoyl chloride	0.53
7	25.683	Aminocyano acetic acid	1.03
8	44.442	Pentadecanoic acid	8.65
9	51.108	Hexanoic acid 2-hydroxy	0.65
10	54.400	Oleic acid	62.82
11	59.333	9-Octadecenoic acid, (E)-	1.68
12	62.075	9-Octadecenamide, (Z)-	6.19
13	67.317	2, 4-Bis(dimethylbenzyl)-6-t-butylphen	0.62
14	68.192	Pentacosane	0.63
15	72.117	Hexacosane	0.70
16	74.050	Cholesterol	10.84
17	75.900	Heptacosane	1.06

Table (4): Infra red spectrophotometery data

Frequency(cm-1)	Type of vibrate	Assignment
3600 – 2400	O-H	H_2O
1680 – 1600	C=O	CaOH group
1600 – 1500	C-C	Aromatic
1448 – 1097	NH	NH_2
1322	-S=O	SO_2 group
582	CI	CI

Table (5): Medicinal Uses of Some Degraded Compounds in Female Camel Urine

Compounds	Formula	Medicinal Uses	References
Cycloserine	$C_3H_4N_2O_2$	Antibacterial , Tuberclostatic	Merck Index (2006)
Caprylate	$C_{16}H_{30}O_4SI$	Fungicide	"
Hexadecanoic Acid	$C_{16}H_{32}O_2$	Sclerosing agent	"
Stearic acid	$C_{18}H_{36}O_2$	Suppositories enteric coating	"
Tetradesanoic Acid	$C_{18}H_{28}O_2$	Cosmotic Ingredient In Soap And Shaving	"
Pthalic Anhydride	$C_8H_4O_3$	Antificial Resins	"
2Hydroxy Cyclo Decanone	$C_{10}H_{18}O_2$	Used as mucolytic	"
Oleic acid	$C_{36}H_{36}O_2$	Diagnostic aid in pancreatic function	"
Dodecmethylpenta siloxane	$C_{12}H_{36}O_5Si_5$	Withstand heat extremities	"
4 Syclododcyle-2-6 dimethyl morphine	$C_{18}H_{35}No$	Fungicide	"
E-9-Octa decanoic acid	$C_{18}H_{34}O_2$	Choleratic lupricating oil	Merck Index 68,98,06

Conclusion

Medicinal uses of some of these compounds, confirm the therapeutic effects of Female Camel Urine in our previous clinical studies .To enhance the utility and convenience of the degraded compounds, each compound should be fractionated and monitored to know its bioactivity against the actual disease.

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