Gas chromatography mass spectrophotometry (gc-ms) analysis of female camel urine extracts

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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 verify that female camel urine extracts are an 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 identified profound medical uses (Christy, 2000: Peroni, 2001: Natalie, 2002).The use of urinary remedies to deal with illness has been gaining high popularity in Asia (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", and especially postpartum – 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 (Natalie, 2002). The reliable therapeutic efficacy obtained from clinical studies on camel urine is recorded by (Ohag, 1993, 1998); Kabariti,1988; Burziski,1977); (Mona, 2003); (Wisal, G. 2002); (Salwa *et al*, 2006). These experiments showed that camel urine contains many complex bioactive compounds which can act against bacterial, fungal, viral, parasitic and carcinogenic agents, and it has the ability to protect the liver against toxic agents (Salwa *et al*, 2009). Ethnopharmacology of camel urine as a folk medicine has been gaining popularity for a variety of ailments, particularly in the light of significant advances that have been made in recent years. The methods of pharmacology-pharmochemistry and chromatography offer insights in the etiology and discovery of new drugs and compounds. However there is a continuing need for a vast improvement in our knowledge and understanding of discovering new compounds and drugs. In what follows I identify and summarise significant advances with regard to medicinal compound in female camel urine.

Objectives:

The known therapeutic efficacy of female camel urine and its extracts led us to analyze and investigate its bioactive chemical components.

Materials and methods:

Sample collection:

Female camel urine was collected by natural urination or by *tashweel* technique. Using sterile containers.

Sample preparation:

Chloroformic Extract:

Equal volumes of female camel's urine (FCU) and chloroform were shaken for three hours and left to separate; the lower chloroformic layer was then displaced and analyzed by GC-MS

Ethanolic Extract

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

Lyophilized Female Camel Urine

Two ml of female camel urine were poured into piqué bottles and freeze dried by freeze drier machine.

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. 1 ml of the given sample was 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 conducted 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. Figure 1 and table 1 represent the GC-MS chromatogram of lyophilized urine. Figure 2 and table 2 for ethanolic extract degraded compound; Figure 3 and table 3 showed the chromatogram and degraded compounds of chloroformic extract. Figures 4 and 5 represents the infrared (IR) analysis of lyophilized and chloroformic extract of female camel urine. Tables 4 and

5 showed 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 comparable degraded compounds. These compounds contain aliphatic hydrocarbon 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 suggested 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); (Ohag, 2010). The uses of camel urine as antibacterial, antifungal, antiparasitic, and as an ingredient for cosmetics were reported by (Christy,2000; Natalie,2002); degraded compounds were confirmed 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.

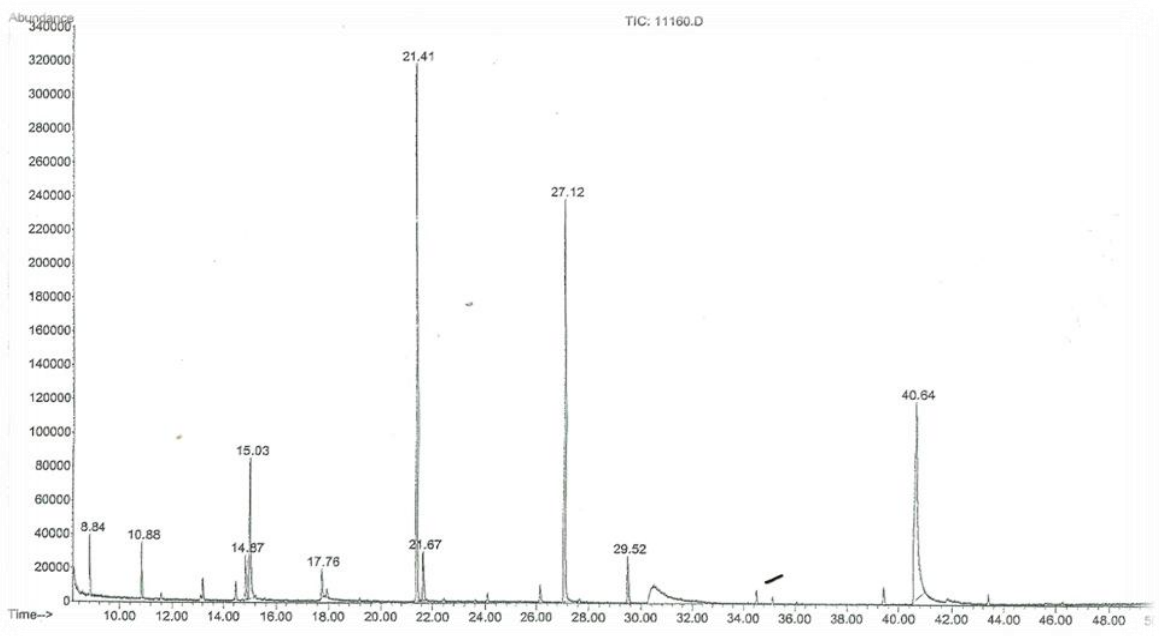

Fig (1) Gas chromatography mass spectrophotometer chromatogram of lyophilized female camel urine

Table (1): GC-MS degraded compounds of lyophilized female camel urine

Peak	R.T.	Compounds	(b)Match	(a)%of tota
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	Benzeneacetic acid, 2-methylpropyl ester	866	2.67
11	40.64	Butylparaben	914	27.71

(a) The lowest % reported is 0.7% - any thing lower than 0.7% was omitted
(b) The chromatogram was matched with NIST library - if a match is more than 800, then probably the compound is preasent

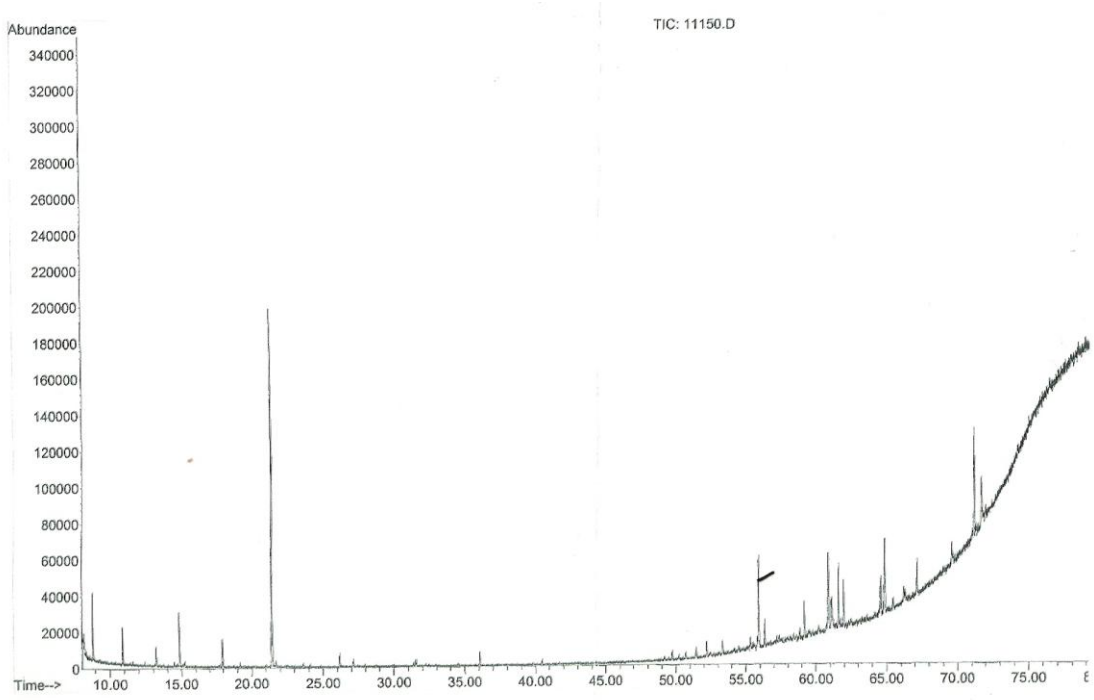

Fig (2): Gas chromatography mass spectrophotometer chromatogram of ethanol extract of female camel urine

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-Heptanone, 3-methyl-	867	3.82
2	10.89	Butanoic acid, butyl ester	851	2.41
3	14.88	Acetic acid, [(2,4,6-triethylbenzoyl)thio]-	815	3.55
4	17.96	Sulfone, 2-hydroxybutyl t-butyl	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]-	699	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	Octadecanoic acid, 2-methylpropyl ester	734	5.23
12	62.00	Heptacosane	689	3.84
13	64.65	Heptacosane	607	3.65
14	64.92	2,4-Bis(dimethylbenzyl)-6-t-butylphenol	575	8.23
15	67.2	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 ester, (Z,Z,Z)-	609	5.32

*(a) The lowest % reported is 0.7% - any thing lower than 0.7% was omitted
*(b) The chromatogram was matched with NIST library - if a match is more than 800, then probably the compound is preasent

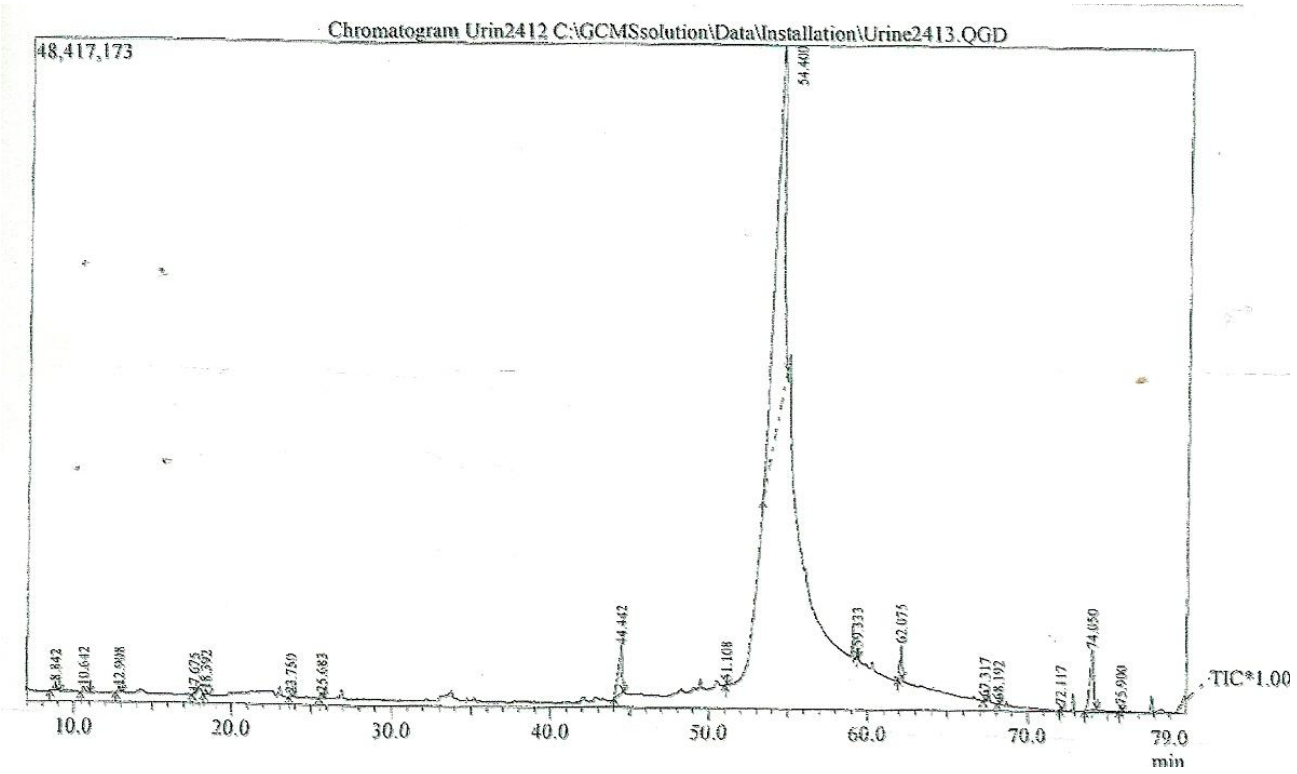

Fig (3): Gas chromatography mass spectrophotometer chromatogram of chloroform extract of female camel urine

Table (3): GC-MS degraded compounds of chloroform extract of female camel urine

Peak Report TIC

Peak#	Name	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H
1	Heptanoic acid	8.842	8.533	9.133	5632236	0.47	655611	1.59	8.59
2	Phthalic anhydride	10.642	10.450	11.075	5281298	0.44	414299	1.01	12.75
3	n-decanic acid	12.908	12.708	13.142	3240784	0.27	390164	0.95	8.31
4	Benzoic acid	17.675	17.542	17.783	261327	0.02	85358	0.21	3.06
5	Cyclooctanone	18.392	18.258	18.608	3077224	0.26	344381	0.84	8.94
6	Dodecanoyl chloride	23.750	23.625	23.875	1414180	0.12	217615	0.53	6.50
7	4-aminocinnamic acid	25.683	25.517	25.850	4239365	0.35	424276	1.03	9.99
8	Pentadecanoic acid	44.442	43.992	44.608	54726928	4.57	3555151	8.65	15.39
9	Hexanoic acid 2-hydroxy	51.108	51.042	51.183	1182400	0.10	267732	0.65	4.42
10	oleic acid	54.400	53.250	54.725	1016040065	84.84	25826037	62.82	39.34
11	9-Octadecenoic acid, (E)-	59.333	59.275	59.417	2858240	0.24	692303	1.68	4.13
12	9-Octadecenamide, (Z)-	62.075	61.892	62.175	21293881	1.78	2543503	6.19	8.37
13	2,4-Bis(dimethylbenzyl)-6-t-butylphen	67.317	67.233	67.408	1188374	0.10	255325	0.62	4.65
14	Pentacosane	68.192	68.108	68.275	1224754	0.10	259163	0.63	4.73
15	Hexacosane	72.117	72.050	72.192	1283030	0.11	286209	0.70	4.48
16	Cholesterol	74.050	73.600	74.350	72224983	6.03	4456902	10.84	16.21
17	Heptacosane	75.900	75.800	76.000	2496480	0.21	435385	1.06	5.73
					1197665538	100.00	41109414	100.00	

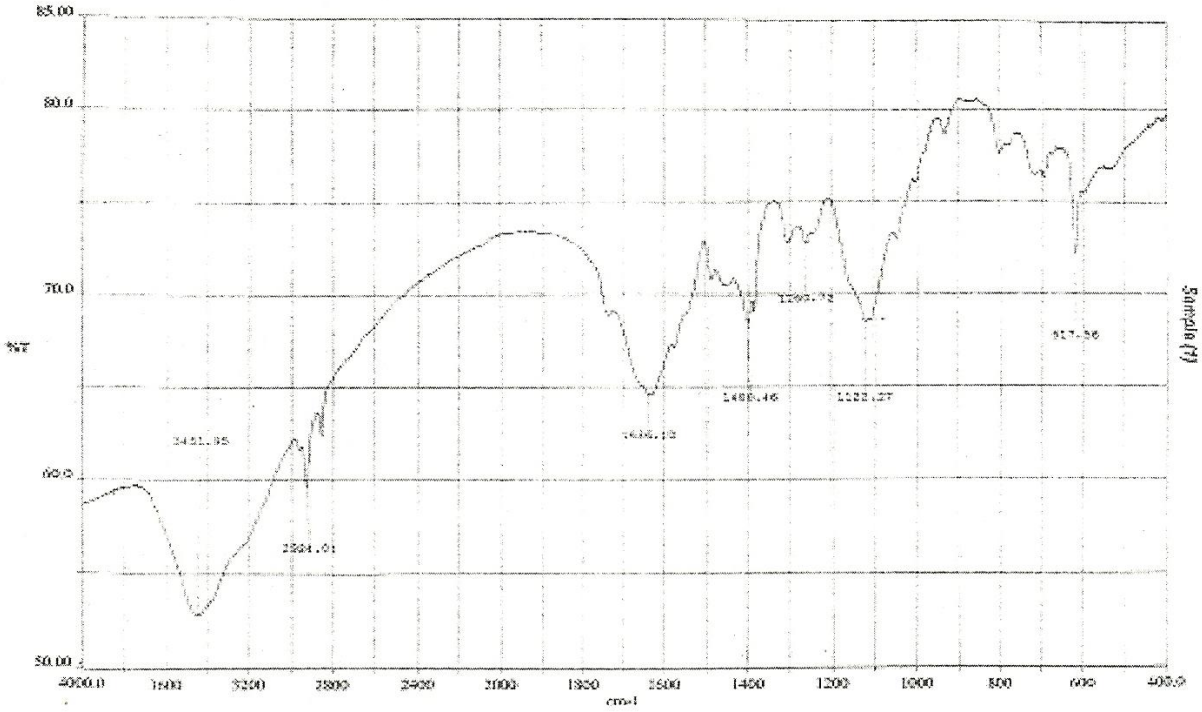

Fig (4): Infrared spectrophotometer of chloroform extract of female camel urine

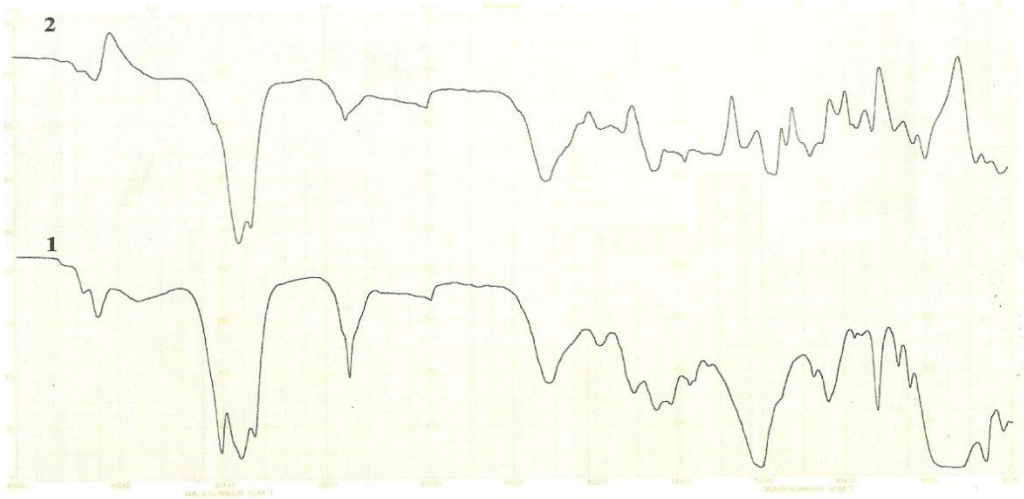

Fig. (45 a)
Infrared spectra of chloroformic extract from Camel urine .
key 1) - chloroform ; 2) pure sample of extracted componant (s)

Table (4): Infrared spectrophotometry 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	$C_{18}H_{28}O_2$	Cosmetic 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 lubricating 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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REFERENCES:

Armstrong, J.W. (1971) *The Water Of Life*: *A Treatise on Urine Therapy,* Health Science Press, Rustington, UK.

Burzynski, Stanislaw. R. *et al* (1977) "Anti neoplaston A in cancer therapy", *Physiology, Chemistry and Physics*, vol. 9, 485.

Christy, M. Martha, (2000) *Your Own Perfect Medicine*

Kabariti, A., Mazruai, S. and Elgendi, A. (1988) "Camel urine: A possible anti carcinogenic agent", *Arab Gulf. J.Sci, Res.Agric, Biol.Sci.*

Mona, A. Khalifa (2003) "Antibacterial effects of camel urine (*Camelus dromedarius*)", MVSc dissertation, University of Khartoum, Sudan.

Natalie, B. (2002) "Urine therapy (drinking urine)". *J. of Berkeley Medicine*.

Ohaj, H.M. (1993)"Camel urine as medicament in Sudan", BSc dissertation, University of the Gezira, Sudan.

Ohag, H.M. (1998) "Clinical trials for treatment of ascites with camel urine", MSc. University of Gezira, Sudan.

Pieroni A., A.Grazzini and M.E.Giusti (2002) "From the sources of knowledge to the medicines of the future", *Proceedings of the 4th European Colloquium on Ethnopharmacology,* IRD Editions, Paris, France, pp. 371-5.

Salwa, M/E., Khogali; O.Y. Mohamed; A.M. Elhassan and A.M.A. Magid (2006) "Therapeutic applications of she-camel urine: pathological changes in cattle infected with fasciolosis". *Albuhuth*, vol 10 (1):109-122.

Salwa, M/E., Khogali; O.Y. Mohamed; A.M. Elhassan; A.M. Shammat and A.M.A. Magid (2009) "Hepatoprotective effect against carbontetrachloride induced hepatotoxicity in rats", *J.SCi.and Techn*., vol.10 (2):128-34.

Khorshid, F.A., Moshref, S.S.; Heffny, N. (2005)"An ideal selective anticancer agent in vitro, 1-tissue culture study of human lung cancer cells A590", *JKAU-Medical Sciences*, vol. 12, pp. 3-18.

Wisal, G.A. (2002) "Antibacterial and antifungal effect of camel urine (*Camelus dromedarius*)", MVSc dissertation, University of Khartoum, Sudan.

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