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A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN HERBAL MEDICINAL PREPARATIONS: A QUEST FOR SAFETY FROM CARCINOGENS

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ABSTRACT The use of medicinal plants in primary healthcare is increasing worldwide and there is need to determine the safety of herbal medicinal preparations obtained from these plants. Due to the increase in cancer cases worldwide it is expedient to monitor carcinogens in herbal medicinal preparations sold to the public**.** ***Objective***: The objective of this study was to determine the levels of PAHs in Boswellia *serrata*, Prosopis *africana*, Anogeissus *leiocarpus* and Sclerocarya *birrea* herbal medicinal preparations obtained from the Markets in Zaria, Kaduna State. The United States Environmental Protection Agency (US EPA) Method 3550 for the extraction of non-volatile and semi –volatile organic compounds from solid samples was used. US EPA method 8310 was employed in the separation of 18 PAHs on Phenomenex Luna C18 (250 x 3.0 mm, 5 µm) column and quality control measures were undertaken to determine method performance, sensitivity, accuracy and precision of the method used. Phenanthrene was detected in stem barks of *Prosopis africana* and *Sclerocarya birrea* at concentration of 0.0625 mg/kg and 0.0648 mg/kg respectively. Pyrene was detected in stem barks of *Anogeissus leiocarpus*, *Prosopis africana* and *Sclerocarya birrea* at concentrations of 0.0.0091 mg/kg, 0.0137 mg/kg, and 0.027mg/kg respectively. The sum of PAHs in stem barks of *Anogeissus leiocarpus*, *Prosopis africana*, *Sclerocarya birrea* and *Boswellia serrata* was 0.0941 mg/kg, 0.0762 mg/kg, 0.0855 mg/kg and 0.00mg/kg respectively. The herbal medicinal preparations monitored in this study had the ∑PAH below 2.0 mg/kg WHO permissible limit which makes the substances analysed safe for human consumption.

Key words: Carcinogenic, Herbal medicinal preparations, PAH, RP-HPLC, safety,

INTRODUCTION:

Herbal medicinal preparations are gaining popularity throughout the world and the global trade as at 2019 was over US$129 billion (https://www.marketresearchfuture.com/sample). It has been estimated that 80% of people world over rely on herbal medicines for primary healthcare; those who take herbal medicines outnumber those who take Orthodox medicines by about two to three times (WHO, 1996; Ekor, 2013). The clamour for the use of herbal
medicines stems from the fact that herbal medicinal preparations are readily available, cheap and thought to be safer than Orthodox medicines (WHO, 1996). Due to the popularity of herbal medicinal preparations in the primary healthcare system of especially under developed countries of Africa and Asia, they have to be monitored for carcinogens and other toxic substances, because cancer is on the increase worldwide, with estimated number of people to have come down with cancer by 2020 placed at 30 million (Sloczynska *et al.*, 2014).

Food and herbal medicinal preparations should be monitored, because they can be contaminated by various contaminants, which may include bacteria, fungi, heavy metals and polycyclic aromatic hydrocarbons found in the environment. Polycyclic aromatic hydrocarbons are ubiquitous and persistent organic compounds with high molecular weights and low volatility at room temperature. They comprise of over 100 different chemicals that consist of two or more fused benzene rings in linear, angular or cluster arrangements (Skupinska *et al.*, 2004).

Much research has been done on bioactive constituents of herbal medicinal preparations used in the treatment of human and animal ailments, but less work on the safety of these substances in terms of

both environmental organic pollutants, PAHs in particular and carcinogenic compounds in general.

The contamination of food and herbal medicinal preparations by PAHs could be from the environment, industrial processes or through some cooking practices that may involve the production of PAHs through incomplete combustion or pyrolysis of organic matter (EFSA, 2008). The safety of food has been the concern of many world bodies such as International Programme on Chemical Safety (IPCS), Food and Agriculture Organization / World Health Organization (FAO/WHO) and the Joint Expert Committee on Food Additives (JECTA).The concern about the safety of food spurred the evaluation of PAHs in foods by the IPCS in the last ten years.

The Scientific Committee on Food (SCF), FAO/WHO and JECTA also joined the IPCS evaluation; they adopted the 15 PAHs selected by SCF that showed clear evidence as being mutagenic and genotoxic in somatic cells in *in vivo* animal experiments (EFSA, 2008).

For this reason, the SCF has reasoned and regarded these as potential genotoxic and carcinogenic substances to humans and therefore represent a priority group in the assessment of the risk of long term adverse health effects resulting from daily dietary intake of PAHs (EFSA, 2008). As a result of the examination of PAHs profile in food and the study that evaluated the carcinogenicity of the two coal tar mixtures in mice, the SCF suggested that benzo (a) pyrene be used as a marker for the occurrence and toxicity of PAHs in foods.

Due to further investigation into the levels of PAHs in certain foods as recommended by the European Committee (EC) in Regulation 2005/108/EC, the eight member States of the EC submitted 10,000 results for PAHs levels in different food commodities. The evaluation of these results by EFSA Panel on Contaminants in Food Chain (CONTAM panel) in June 2007 and updated in June 2008 demonstrated that benzo (a) pyrene could be detected in about 50% of the samples. However, in about 30% of the samples in which benzo (a) pyrene was not detected, other carcinogenic and genotoxic PAHs were detected. Chrysene was the most commonly found PAH in the food samples tested. It was in view of these findings that the EFSA Panel on Contaminants in Food chain (CONTAM panel) reviewed the available data on occurrence and toxicity of PAHs (EFSA, 2008) and added benzo (c) fluorene as suggested by JECFA, (2005). In this view, special attention was paid to those eight carcinogenic and genotoxic PAHs namely benzo (a) pyrene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (g, h, i) perylene, chrysene, dibenz (a,h) anthracene and indeno (1, 2, 3 –cd) pyrene) that were measured in the two coal tar mixtures used in the carcinogenic tests by Culp *et al*.(1998).

This study determined the levels of PAHs in herbal medicinal preparations obtained from stem barks of *Anogeissus leiocarpus, Boswellia serrata, Prosopis africana* and *Sclerocarya birrea* herbal medicinal preparations sold in markets of Zaria by Reverse – phase high performance liquid chromatography (RP-HPLC) as a means of quality control. The results would be used to create awareness on the safety of these herbal medicinal preparations and their plants.

MATERIALS AND METHODS

Materials

The materials consisted of ultrasound sonicator, water bath, nitrogen gas, dichloromethane, acetonitrile and water. All chemicals were of HPLC grade. Mixture of 18 PAHs calibration continuous verification standard and laboratory control standard coded (CCV10#9A23039 and LCS (B9E2315-BS1) respectively; matrix spike and matrix spike duplicate and blank all obtained from Phoslab Environmental Services..

Methods

Sample Collection

Four powdered herbal medicinal preparations obtained from stem barks of *Anogeissus leiocarpus, Prosopis africana, Boswellia serrata* and *Sclerocarya birrea* were randomly purchased from the markets of Zaria. The samples were put into boiling tubes, packaged in a cold chain before analysis.

Extraction of polycyclic aromatic hydrocarbons from herbal medicinal preparations

United States Environmental Protection Agency (US EPA) 3550 C a method of extracting volatile and semi-volatile organic substances from plant tissues using ultrasonic sonication was used. The technique uses ultrasound to facilitate the transfer of analyte into the extraction solvent through the cavitation phenomenon where micro-bubbles form and collapse as soon as they are formed (Oluseyi 2011). The extracted samples were cleaned using commercially available syringe barrel packed with florasil and the volume of the extract reduced to about 1ml by evaporation over hot water bath set at 100 ^{o}C and exchanged with acetonitrile.

Recovery experiment

Quality control measures were adequately carried out through blank analysis to determine the performance of the method through the determination of method detection limit (MLD) and practical quantification limit (PQL).

Surrogate standard (o-Terphenyl) and laboratory control standard (LCS) were added to all samples,

blank, matrix spike, and matrix spike duplicate to determine accuracy and precision of the method.

Instrument and analytical conditions (HPLC Sample Run)

Reverse phase –high performance liquid chromatography (RP-HPLC) with uv/fluorescence detector was used to determine 18PAHs in herbal medicinal preparations obtained from stem barks of *Boswellia serrata*, *Anogeissus leiocarpus*, *Prosopis africana* and *Sclerocarya birrea* plants with binary mobile phase of deionised water and acetonitrile. The Agilent series 1100 equipped with 1100 binary pump (G1312A), 1100 Autosampler (G1313A), 1100 variable wavelength detector (G1314A), 1100 column Thermostat (G1316A) with Phenomenex Luna C18 (250 x 3.0 mm, 5 µm) column, 1100 Fluorescence/uv detector (G1321A) HPLC was used. Instrument parameter settings were ambient temperature, mobile phases A: Deionised water, B: Acetonitrile. The elution was programmed at zero time A=60%, B= 40%, 5 minutes A=60%%, B= 40% at 30mins A=0%, B=100% and at 45mins A=0%, B=100%. The flow rate was set at 1.5ml/min. The volume injected was 6µl and UV detector was set at 254 nm and fluorescence detector was set at 280/389 for excitation/ emission. Data were acquired and analysed with ChemStation Rev. A.08.03 (847) and ChemStation Rev. C.01.09 (144) respectively. HPLC conditions were as stated in Table 1.

RESULTS & DISCUSSION

Validation of analytical procedures adopted

The Retention Times of PAHs obtained from stem barks of *Anogeissus leiocarpus*, *Boswellia serrata*, *Prosopis africana* and *Sclerocarya birrea* herbal medicinal preparations as identified by fluorescence and uv detectors are presented in Table 1. The chromatograms of standard PAHs obtained by both fluorescence detector with Excitation at 280 and Emission set at 389 and uv set at 254 nm are presented in figure 1, where the retention times were obtained. Acenaphthylene was not detected by fluorescence detector because it does not fluoresce. The total ion chromatogram is shown in Figure 1 in which, acenaphthylene, fluorene and indeno (123-cd) pyrene PAHs were not detected by fluorescence detector and acenaphthene was not detected by uv detector. The Retention Times of the PAHs as measured by fluorescence and uv detectors had relative percent standard deviations (%RSD) in the range 0.016 to 0.025.

The analysis of blank (B9E2315-BLK1) in which the method detection limits (MDL) and the practical quantification limits (PQL) of the PAHs analysed were determined are presented Table 2. The Method Detection Limit (MDL) ranged between 0.00300 mg/kg and 0.00800 mg/kg.

The practical quantification limit in B9E2315-BKL1 was 0.0133 mg/kg for all the PAHs analysed by this method (EPA Method 8310). The recoveries of o-Terphenyl from spiked media to adjudge the extraction method are presented in Table 3 Surrogate standard (o-Terphenyl) was spiked into Blank (B9E2315-BKL), LCS B9E2315-MS), Matrix spikes (B9E2315-MS1 and Matrix spike duplicate (B9E2315-MSD1) and extracted by sonication

(EPA method 3550).The recoveries of o-Terphenyl extracted from the spiked media ranged from 83.2% to 90.5 %.The recoveries of standard LCS (B9E2315-BS) spiked into a blank are presented in Table 4. The standard consisted of naphthalene, 2-methyinaphthalene, phenanthrene, pyrene, benzo (a) pyrene and chrysene. The recoveries after analysis using RP-HPLC with EPA method 8310 were naphthalene 96.6%, 2-methylnaphthene 114%, and phenanthrene 85.5%, benzo (a) pyrene 99.0% and chrysene 101%. Sample matrix spiked with naphthalene, 2-methylnaphthene, phenanthrene, pyrene, benzo (a) pyrene and chrysene had recoveries of 79.3%, 92.4%, 81.4%, 94.2%, 114.0% and 106% respectively as presented in Table 5. Sample matrix duplicate spiked with naphthalene, 2-methyinapthene, phenanthrene, pyrene, benzo (a) pyrene and chrysene had recoveries of 82.1%, 90.9%, 81.2%, 92.0%, 115% and 106% respectively as presented in Table 6. The matrix spike and its duplicate had relative percent difference (RPD) of 3.95, 1.17, 0.187, 1.85, 1.28 and 1.08 for naphthalene, 2-methylnaphthalene, phenanthrene, pyrene, benzo (a) pyrene and chrysene respectively

The concentrations of PAHs identified in *Anogeissus leiocarpus*, *Boswellia serrata*, *Prosopis africana* and *Sclerocarya birrea* herbal medicinal preparations are shown in Table 7. The naphthalene concentration in *Anogeissus leiocarpus, Prosopis africana*, *Boswellia serrata* and *Sclerocarya birrea* herbal medicinal preparations was below method detection limit (BMDL).

Acenaphthene, acenaphthylene, anthracene, benzo (ghi) perylene, fluoranthene and fluorene were all below detection limit in all herbal medicinal preparations. Phenanthrene was present only in *Prosopis africana* and *Sclerocarya birrea* herbal medicinal preparations at 0.0625 mg/kg and 0.0648 mg/kg respectively. Pyrene was detected in *Anogeissus leiocarpus*, *Prosopis africana*, *Sclerocarya birrea* and *Boswellia serrata* herbal medicinal preparations at 0.00941mg/kg (this is above MDL in reagent water), 0.0137 mg/kg and 0.0207 mg/kg (these are above PQL). Moreover, all the high molecular weight PAHs consisting of benzo (a) anthracene, benzo (a) pyrene, benzo (b)

fluoranthene and benzo (k) fluoranthene, dibenz(ah) anthracene, benzo (ghi) perylene and indeno(123-cd)pyrene were below method detection limits in *Anogeissus leiocarpus*, *Prosopis africana*, *Boswellia serrata* and *Sclerocarya birrea* herbal medicinal preparations. Chrysene was not detected in any of the samples analysed. The sum of polycyclic aromatic hydrocarbons in herbal medicinal preparations was *Anogeissus leiocarpus* 0.00941mg/kg, *Prosopis africana* 0.0762mg/kg, *Sclerocarya birrea* 0.0855 mg/kg and 0.00 mg/kg in *Boswellia serrata*.

Naphthalene in Table 2 a low molecular weight polycyclic aromatic hydrocarbon was below method detection limit in all the samples of herbal medicinal preparations. It has been reported that naphthalene is a possible carcinogenic compound as listed by IARC, (2006). Naphthalene forms tumours in rodents as reported by Buchholz *et al*., (2019) who determined the ability of naphthalene to form adducts with deoxyribonucleic acid (DNA) by culturing ex-vivo metabolically active lung tissues labelled with carbon -14 with concentrations of naphthalene ranging from 0-250µM. Buchholz *et al.,* (2019) discovered that even with relatively low metabolic bioactivation in primate air pathway, dose- depended naphthalene- deoxyribonucleic acid (NA-DNA) adduct formation was detected. The formation of NA-DNA adduct suggested that NA-DNA may contribute to in-vivo carcinogenesis through a genotoxic mechanism Buchholz *et al.*, 2019)

The reason naphthalene was not detected in the selected herbal medicinal preparations could be due to its low molecular weight, thus it could have volatilized, undergone photolysis (maximum half-live is 12.2hr) (US EPA, 1990) or it was initially not present in the samples analysed. Phenanthrene was detected above method detection limit in *Sclerocarya birrea*, *Prosopis africana* herbal medicinal preparations, but not in *Anogeissus leiocarpus* herbal medicinal preparation. Phenanthrene is one of the low molecular weight PAHs that is thought of as a non-carcinogenic PAH (Philips, 1999). However, phenanthrene is an indirect – acting agent that may require metabolic activation by cellular enzymes (members of cytochrome P450) to form DNA – reactive metabolites (Moorthy *et al*., 2015).

Investigating the presence of 16 EPA PAH in nine Chinese medicinal herbs, phenanthrene was detected in liquorice (631.3µg/kg), indigo wood leaf (551.0µg/kg), rose–flower (435.2µg/kg) and in eucommia barks (432.3µg/kg) (Yu *et al.,* 2012). Comparing the concentration of phenanthrene obtained from *Prosopis africana* (0.0625mg/kg) and Sclerocarya *birrea* herbal medicinal preparations (0.0648 mg/kg) in our study to those by Yu *et al.* (2012), it is noted that their results were about ten times those obtained in our study; this could be due to environmental factors or due to plant species variations. Pyrene was detected in *Anogeissus leiocarpus* above method detection limit in reagent water at concentration of 0.00941mg/kg, *Prosopis africana* (0.0137mg/kg) and Sclerocarya *birrea* herbal medicinal preparation (0.0207mg/kg) which are above practical quantification limit of the method of analysis.

The concentration of pyrene in *Sclerocarya birrea* herbal medicinal preparation was about two times that in *Anogeissus leiocarpus* and one half of what was in *Prosopis africana.* Chukwujindu *et al.,* (2015) investigated the concentrations of PAHs profile in some Commercial Brands of Tea-, Coffee-, and Cocoa-Based Food Drinks in Nigeria and their results showed that the concentration of pyrene in teas ranged between not detected to 224 µg/kg; the highest concentration obtained in TB8 brand of tea was about ten times the concentration of pyrene in *Sclerocarya birrea* herbal medicinal preparation in the present study. The sum of PAH in herbal medicinal preparations ranged between 0.0 mg/kg in *Boswellia serrata* herbal medicinal preparation and (0.0855mg/kg) *Sclerocarya birrea* herbal medicinal preparation. Yu *et al.* (2015) reported the sum of PAHs in 5 teas and 29 medicinal plants in the range of 0.0065mg/kg and 1,112mg/kg in Eucommia bark, while the sum of PAHs in Chinese herbal medicines varied from 0.0982 mg/kg in cassia seed to 2,24mg/kg in Eucommia bark.

Krajian and Odeh (2013) determined the concentrations of PAHs in leaves and flowers of medicinal plants and values recorded were in the range 0.0470 mg/kg to 0.980 mg/kg in sage plant. The minimum value of 0.0470 mg/kg obtained in sage plant by Krajian and Odeh was about half the value of PAHs obtained (0.0855mg/kg) in *Sclerocarya birrea* herbal medicinal preparation in this study. The differences in results recorded by Yu *et al.,* (2015), Krajian and Odeh (2015) and the result in this study may be due to environmental factors (soil, water or proximity to urban area) or methods of preparing these plant materials (drying and or smoking) or could be due to plant species variations.

CONCLUSION

The sums of PAH in the herbal medicinal preparations analysed were below 2.0 mg/kg WHO permissible limit thus, it could be said that, the substances analysed were safe for human consumption in respect to of PAHs concentrations.

RECOMMENDATIONS

Even though, this study indicated that the herbal medicinal preparations analysed may be safe for human consumption in respect to PAHs, none the

less, it is recommended that confirmatory analysis be done using another method say GC/MS and monitoring exercise of the substances should be continuous. Also, the substance should be monitored for other toxic substances

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interest to declare

REFERENCES

Buchholz A.B., Carratt, S., Collette M. N., Kuhn, A.E., Winkle V.,S.L., Ding X 2019 Naphthalene DNA Adduct Formation and Tolerance in Lung *Nuclear Inst. and Methods in Physics Research B:Beam interactions with materials and atoms vol438, 119-120*

Chukwujindu, I., Tesi, G., Bassey, I. F., Matincigh, B., Nwajei, E G 2019.Determination of polycyclic Aromatic Hydrocarbons in Water and Gin-Based Tea Infusions of Selected Tea Brands in Nigeria. *Polycyclic aromatic compounds* 36(4):1-23

Culp, S.J., Gaylor, D.W., Winslow, G.S., Goldstein L. S, Beland, F.A. 1999 A Comparison of the Tumour Induced by Coal tar and Benzo (a) pyrene in a 2-year Bioassay *Carcinogenesis 19(1):117-124*

Ekor, M., 2013.The Growing use of Herbal Medicines Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. *Frontiers in Pharmacology* 4: 177.

European Food and Safety Journal (EFSA Journal, 2008) polycyclic aromatic hydrocarbons in Food.*EFSA, Journal, vol.724:1-114* http://www.atsdr.cdc.gov/toxfaq/tf.asp?d=p

https://www.marketresearchfuture.com/sample retrieved august 11, 2021 4.pm

IARC International Agency for Research on Cancer 2006. IARC Definition and Compound list. Preamble to IARC Monographs vol. 75 (Amended January 2006).

Krajian H. and A. Odeh 2013. Polycyclic Aromatic Hydrocarbons in Medicinal plants from Syria. *Toxicological Environmental chemistry* vol. 95 (6): 942 – 953.

Moorthy, B., Chu C., and Cartin, J. D. 2015 Polycyclic Aromatic Hydrocarbons: From metabolism to Lung Cancer. *Toxicological Sciences 145(1):5-15.*

Oluseyi T, Olayinka, K., Ola B., and Smith R.M 2011 Comparison of Extraction and Clean-up Techniques for Determination of Polycyclic Aromatic Hydrocarbons in Contaminated Soil Samples. *African Journal of Environmental Science and Technology vol.5 (7): 482-493*

Phillips D.H. 1999. Polycyclic Aromatic Hydrocarbons in the diet. *Mutation Research*, 443 (1-2):139–147.

Skupinska K, Misiewicz I. Kasprzcka-Guttmam T.2004). Polycyclic Aromatic Hydrocarbons: Physicochemical Properties, Environmental Appearance and Impact on Living Organisms. *Acta Poloniae Pharmaceutica –Drug Research*, 61 (3) 233-240.

Sloczynska, K., Powroznik, B, Pekala, E. and Waszkielewicz, A.M. 2014. Antimutagenic Compounds and Their Possible Mechanism of Action. *Journal of Applied Genetics* 55(2): 273-285

US EPA (United States Environmental Protection Agency 1990 Ambient Water Quality for Polycyclic Aromatic Hydrocarbons. EPA 440/5-80-069.US NTIS PB91-117806. US EPA, Washington, D.C

WHO, 1996. Final report of the seminar on the use of medicinal plants in healthcare of Medical Plants in Healthcare

Yu L., Cao, Y; Jing – Yuan Zhang, & Cui, Z. and Sun H.2014). Isotope dilution – GC/MS analysis of 16 polycyclic aromatic hydrocarbons in selected medicinal herbs use as health food additives, food additives and contaminants – part a chemistry, analysis, control, exposure and risk assessment 29 (11): 1800-1809.

Table 1: The retention times (minutes) of standard (CCV10#9A2339) as recorded by fluorescence and UV detectors

PAHs	Fluorescence Ex280Em:289	UV254	Standard deviation	%Relative standard deviation
Naphthalene	14.190	14.166	0.017	0.001
Acenaphthylene	-	15.376	-	-
1-methylnaphthalene	16.515	16.491	0.017	0.001
2-methylnaaphthalene	16.800	16.778	0.016	0.001
Acenaphthene	17.601	-	-	-
Phenanthrene	18.189	18.164	0.018	0.001
Anthracene	18.767	18.740	0.019	0.001
Fluoranthene	20.114	20.088	0.018	0.001
Fluorene	-	17.346	-	-
Pyrene	20.795	20.769	0.018	0.001
o-Terphenyl	21.372	21.367	0.004	0.001
Chrysene	22.389	22.354	0.025	0.001
Benzo(a) anthracene	22.603	22.569	0.024	0.001
Benzo(b)fluoranthene	24.499	24.471	0.019	0.001
Benzo(k)fluoranthene	24.728	24.698	0.023	0.001
Benzo(a)pyrene	25.218	25.192	0.018	0.001
Dibenz(ah)pyrene	26.090	26.063	0.019	0.001
Indeno(123-cd)pyrene	-	27.049	-	-
Benzo(ghi)perylene	27.310	27.274	0.025	0.001

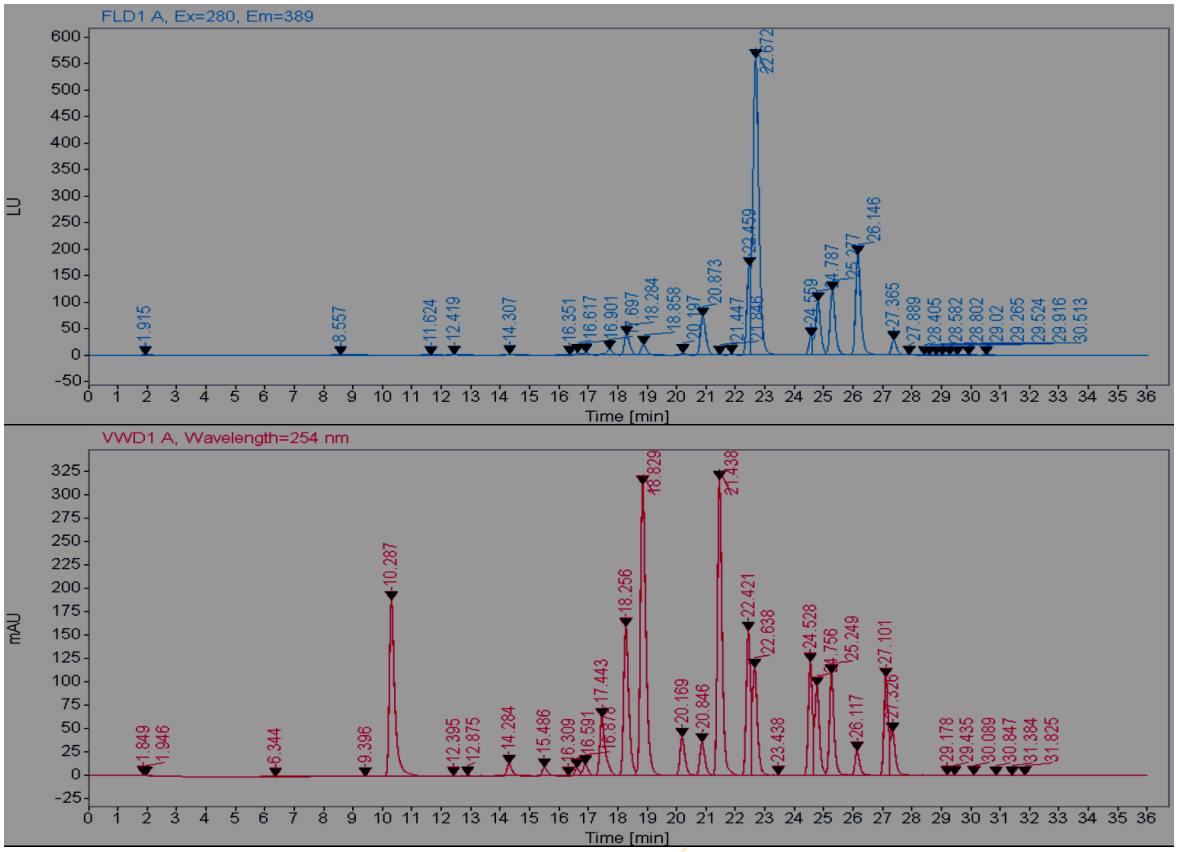

Figure1: Chromatogram of 18 Standard PAHs obtained by RP-HGPLC using fluorescence / uv detectors: Peaks in lower chromatogram are peaks detected by uv at 254 nm. The peaks are; naphthalene-14.166min, acenaphthylene-15.376min, 1-methylnaphthalene-16.491min, 2-methylnaphthalene-16.778min, phenanthrene-18.164min, anthracene-18.740, fluoranthene-20.088min, pyrene-20.769min, o-Terphenyl-21.367min, chrysene-22.354min, benzo (a) anthracene-22.569min, benzo(b)fluoranthene-24.471min, benzo(k)fluoranthene-24.698min, benzo(a)pyrene-25.192min,.dibenz(ah)pyrene-26.063min, indeno!,2,3-cd)pyrene-27.049min, benzo(ghi)perylene-27.274min

Table 2: Concentrations of PAHs in Herbal Medicinal Preparations

PAH	SAMPLE ID (mg/kg)			
	Anogeissus leiocarpus herbal medicinal preparation	*Prosopis africana* herbal medicinal preparation	Sclerocarya birrea herbal medicinal preparation	*Boswellia serrata* herbal medicinal preparation
Naphthalene	Bdl	bdl	bdl	Bdl
Acenaphthene	Bdl	bdl	bdl	bdl
Acenapthylene	Bdl	bdl	bdl	bdl
Anthracene	Bdl	bdl	bdl	bdl
Benzo(ghi)perylene	Bdl	bdl	bdl	bdl
Fluoranthene	Bdl	bdl	bdl	bdl
Fluorene	Bdl	bdl	bdl	bdl
Phenanthrene	Bdl	0.0625	0.0648	bdl
Pyrene	0.00941	0.0137	0.0207	bdl
Benzo(a) pyrene	Bdl	bdl	bdl	bdl
Benzo(a) anthracene	Bdl	bdl	bdl	bdl
Benzo((b) fluoranthene	Bdl	bdl	bdl	bdl
Benzo(k)fluornthene	Bdl	bdl	bdl	bdl
chrysene	Bdl	bdl	bdl	bdl
Dibenz(ah) anthracene	Bdl	bdl	bdl	bdl
Indeno(123-cd) pyrene	Bdl	bdl	bdl	bdl
Sum	0.00941	0.0762	0.0855	0.00

Key: bdl= below detection limit

PAHs	Concentration (mg/kg)	MDL	PQL
Naphthalene(PAH2)	0.00600	0.00600	0.0133
1-methylnaphthalene	0.00700	0.00700	0.0133
2-methylnaphthalene	0.00500	0.00500	0.0133
Acenaphthene	0.00800	0.00800	0.0133
Acenaphthylene	0.00500	0.00500	0.0133
Anthracene	0.00300	0.00300	0.0133
Benzo(ghi)perylene	0.00500	0.00500	0.0133
Fluoranthene	0.00400	0.00400	0.0133
Fluorene	0.00500	0.00500	0.0133
Phenanthrene	0.00400	0.00400	0.0133
Pyrene	0.00700	0.00700	0.0133
Benzo(a) pyrene	0.00500	0.00500	0.0133
Benzo(a) anthracene	0.00400	0.00400	0.0133
Benzo(b) fluoranthene	0.00400	0.00400	0.0133
Benzo(k) fluoranthene	0.00300	0.00300	0.0133
Chrysene	0.00400	0.00400	0.0133
Dibenz(ah) anthracene	0.00400	0.00400	0.0133
Indeno(123-cd)pyrene	0.00500	0.00500	0.0133

Table 3: Concentrations of PAHs in Blank (B9E2315-BLK-1)

MDL= Method Detection Limit
PQL = Practical quantification Limit

Table 4: Recoveries of o-Terphenyl spiked in different media

Surrogate: o-Terphenyl	Spike level (mg/kg)wet	Recovered level(mg/kg) wet	% Recovery	% Recovery limits
Blank	6.66	5.65	84.8	70-130

LCS(B9E2315-BS1)	6.66	6.03	90.5	70-130
Matrix Spike (B9E2315-MS1)	66.3	55.20	83.2	70-130
Matrix Spike (BE92315-MSD1)	66.6	55.20	83.6	70-130

Table 5: Recoveries of PAHs Standard LCS (B9E2315-BS-1) from matrix spike

PAH	Spike level	Recovered level(mg/kg)	% Recovery	% Recovery limits
Naphthalene	0.333	0.322	96.6	70-130
2-methylnaphthalene	0.333	0.380	114	70-130
Phenanthrene	0.333	0.285	85.5	70-130
Pyrene	0.333	0.330	99.0	70-130
Benzo(a) pyrene	0.333	0.314	94.0	70-130
Chrysene	0.333	0.337	101	70-130

Table 6: Recoveries of PAHs standard from matrix spike (B9E2315-MS1)

PAH	Spike level	Recovered level(mg/kg)	% Recovery	% Recovery limits	MDL	PQL
Naphthalene	3.33	2.63	79.3	70-130	0.0597	0.132
2-methylnaphthalene	3.33	3.06	92.4	70-130	0.0498	0.132
Phenanthrene	3.33	2.70	81.4	70-130	0.0398	0.132
Pyrene	3.33	3.12	94.2	70-130	0.0697	0.132
Benzo(a) pyrene	3.33	3.79	114	70-130	0.0498	0.132
Chrysene	3.33	3.57	106	70-130	0.0398	0.132

MDL= Method Detection Limit

PQL = Practical Quantification Limit

Table 7: Recoveries of standard PAHs from matrix spike duplicate (B9E2315-MSD)

PAH	Spike level	Recovered level (mg/kg)	% Recovery	% Recovery limits	MDL	PQL	RPD
Naphthalene	3.33	2.74	82.1	70-130	0.0597	0.132	3.95
2-methylnaphthalene	3.33	3.03	90.9	70-130	0.0498	0.132	1.17
Phenanthrene	3.33	2.71	81.2	70-130	0.0398	0.132	0.187
Pyrene	3.33	3.07	92.0	70-130	0.0697	0.132	1.85
Benzo(a) pyrene	3.33	3.84	115	70-130	0.0498	0.132	1.28
Chrysene	3.33	3.53	106	70-130	0.0398	0.132	1.08

MDL= Method Detection Limit
PQL = Practical Quantification Limit