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COMPARATIVE STUDY OF TWO CLASSICAL EXTRACTION METHODS BY USING GC/MS OF *TEPHROSIA NUBICA* BOISS GROWING IN SAUDI ARABIA

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ABSTRACT

A comparative study was done at first time from the aerial parts of *T. nubica* Boiss growing in S. A. by using two conventional extraction techniques Macerated (MA) & Soxhlet (SX) and analyzed by GC & GC/MS. In this work, we compare the extraction effectiveness of two methods commonly applied in lab. Also, a comparison of extracting solvents was performed in current study. The extraction conditions might not be maximized for these two techniques; the select of extraction parameters were depend on the experience from pervious investigation published worldwide. This study focused on the vast variation of the extraction quantities and quality with MA & SX methods. The GC & GC/MS chromatographic analysis results found have observed that the alcohol can give the highest recoveries when compared to other extraction solvents. The comparison made between the MA and SX extraction methods has found that MA is a suitable alternative to SE for the hexane and chloroform chemical composition, but SX did not give a good recovery by these two solvents. This study is the first report describing comparison between MA and SX by using GC & GC/MS.

KEYWORDS: *T.nubica*, SX, Maceration, GC&GC/MS.

INTRODUCTION

Genus Tephrosia F. Fabaceae (Papilionaceae) are contained 400 species. This genus is widely distributed in tropical and sub-tropical regions wide world **(Willis, 1973; Al-Zahrani, 2007)**. Represented in Saudi Arabia by eight species; *Tephrosia heterophylla* Vatke., *T. subtriflora* Hochst., *T. uniflora* Pers., *T. pumila* Lam., *T. purpurea* L., *T. desertorum* Scheele., *T. villosa* L., *and T. nubica* Boiss. **(Chaudhary, 2001).** *T. nubica* is represented by two subspecies which are Ssp. Nubica and Ssp. Arabica (Boiss) found on the west costal region of Saudi Arabia. Traditionally, this genus has been used for the curing of sickliness (rheumatic pains), inflammation, stomach ache, syphilis, abortifacient, respiratory disorders, diuretic and laxative **(Qureshi et al., 2010; Dzenda et al., 2007).** *T. purpurea* is used as tonic, antidiarrheal, leprosy, laxative, antivenom, and antiulcer, **(Virupanagouda *et al*., 2011).**

Tephrosia have been studied for their chemical constituents and pharmacological activities. The main classes of these phytochemicals include rotenoids, flavonoids, sterols, and terpenoids. **(Dagne et al., 2012).** Many compounds were isolated from methanolic extract of the *T. nubica*, such as kaemferol 3,7 dirhamnoside, quercetin 3-galactoside 7-rhamnoside, and quercetin 3-gluctoside 7-rhamnoside. On the other hand, some compounds were isolated from chloroform extract such as semiglabrin, pseudosemiglabrin, Apollinine, and lanceolatin A **(Sharaby and Ammar, 1997).** Many compounds have been studied for their pharmacological and biological activity such as antiplasmodial, anticancer and larvicidal. **(Pirrung and Lee, 1995; Gahlot et al., 2012; Yang et al., 2000; Udeani et al., 1997; Basu et al., 1994; Geetha and Varalakshmi, 2001)**.

There was only one biological studied carried on *T. nubica* Boiss in Egypt. *T. nubica* extracts were tested toward the cotton leafworm leading to serious potential effects on the larvae and adults. They enhanced sterility and decreased level of moth's emergence and pupation which trigger to reduce production of eggs and hatchability **(Sharaby and Ammar, 1997).** Antimicrobial activity of the *T. purpurea, T. tinctoria, T. deflexa, T. linearis* and *T. vogelii* were studied against both gram-negative and gram-positive bacteria **(Wanga et al., 2007, Ganapaty et al., 2008a; Gupta et al., 2008; Chinniah et al., 2009; Annalakshmi et al., 2009; Ganapaty et al., 2010, Kare et al., 2006, Ratsimamanga et al., 1994).** The methanolic extract of *T. purpurea* and *T. tinctoria* showed significant antifungal activity against *A. niger* and *C. albicans* (**Gupta et al., 2008, Ganapaty et al., 2010).** *T. tinctoria and T. pumila* root extracts showed Antiprotozoal and anti-plasmodial activity against *L. donovani, T. b. rhodesiense, T. cruzi* and *P. falciparum* **(Ganapaty et al., 2008b; Ganapaty et al., 2009a).** *T. purpurea* has been reported for antileishmanial activity in hamsters and

Indian monkeys infected by *L. donovani* **(Sharma et al., 2003).**

Many *Tephrosia* species like *T. purpurea T. egregia T. toxicaria* and *T. villosa* have been reported for their antioxidant activit **(Choudhary, 2007; Gunjegaonkar et al., 2010; Arriaga et al., 2009a; Vasconcelos et al., 2009; Prashant and Krupadanam 1993; Kim et al., 2001).** It has been studied that cytotoxic activities in many *Tephrosia* species extracts such as *T. purpurea, T. calophylla, T. candida, T. pulcherrima, T. toxicaria, T. villosa, T. tinctorial, T. vogelii* and *T. pumila* by using different cell lines **(Ganapaty et al., 2009a; Ganapaty et al., 2009b; Roy et al., 1986; Parmar et al., 1988**; **Ganapaty et al., 2009c; Hussain et al., 2012; Shanmugapriya et al., 2011; Subhadra et al., 2011; Nondo et al., 2011; Jang et al., 2003; Ganapaty et al., 2009a**; **Adinarayana et al., 2009**; **Wanga et al., 2007).** *T. purpurea* and *T. villosa* were showed potent antihyperglycemic activity in streptozotocin and alloxan induced diabetic rats respectively **(Pavana et al., 2009; Ahmad et al., 2009; Balakrishnan et al., 2007).** *T. purpurea* possess potent hepatoprotective, antihyperlipidemic and anti-inflammatory activities **(Rajal Shah et al., 2011; Pavana et al., 2007; Akthar et al., 2011; Valli et al., 2011)**. As well as *T. vogelii, T. bracteolata* and *T. sinapou* showed potent anti-inflammatory, antipyretic and analgesic activities **(Adaudi et al., 2009**; **Martinez et al., 2012; Chakradhar et al., 2005).**

T. purpurea, T. vogelii, Aedes aegypti, T. nyikensis and *T. villosa* extracts have been studied for its larvicidal activity against the larvae of *Culex quinquefasiciatus* **(Deepak Kumar et al., 2012**; **Nondo et al., 2011; Vasconcelos et al., 2009; Wanjala et al., 2006).** The different extracts of *T. nubica* were studied against *Spodoptera littoralis* and *Agrotis ipsilon* **(Sharaby and Ammar, 1997**). Phytocompounds such as rotenoid and amorpholone from *T. candida* having significant insecticidal properties **(Kole et al., 1992).**

Most species are studied for their phytocompounds, the majority include flavonoids and rotenoids, but the minority of isolated compounds is few. The genus has significant anticancer and larvicidal potential. Nevertheless, the few detailed about phytochemical and no pharmacological and/or biological studies carried on *Tephrosia nubica* Boiss. In this study, we are focus on the comparative study on chemical composition of two different extraction methods; Soxhlet (SX) and Maceration (MA) by using GC& GC/MS of different extracts of *T. nubica* growing in Saudi Arabia. The performance of an analytical methodology could be improved if a choice extraction procedure is employed which eliminates or decreases interfering compounds in the extract and gives high yield **(Bianco et al., 2008)** For this purpose, a comparison between two extraction methods has been investigated in this work.

MATERIAL AND METHODS

Plant material

The aerial parts of *T. nubica* were collected by Prof. Adnan and Prof. Yusuf in Feb 2014 from south region in the Kingdom of Saudi Arabia. A voucher specimen No. 16244, Box 18 was deposited at the Herbarium of the Pharmacognosy Department, College of Pharmacy, King Saud University.

Solvent

The solvents used were ethanol 95%, hexane 40-60°C, chloroform, ethylacetate, and butanol, which were distilled prior to use. Spectroscopically grade solvents were used for GC/MS analysis while those used for extraction processes were analytical grade.

Soxhlet extraction

SX was performed using about 10.0g spiked soil mixed with 2g anhydrous sodium sulphate (Na2SO4), This mixture was placed in a pre-washed cellulose SX thimble and extracted with 250mL of the selected solvent such as hexane, chloroform, ethylacetate and butanol for 12h at a rate of 7-8 cycles/h, in a pre-washed glass fiber thimble. Extracts were concentrated under reduced pressure using a Buchi® rotary evaporator, model 011, Switzerland. Several studies considered the SX extraction method as benchmark and reference for the evaluation of several other extraction methods (**Sporring et al., 2005; Ahmed et al., 2013**). In the current paper, SX is taken as reference for the evaluation of MA method.

Effect of the choice of extraction solvent

The extraction solvents, time, temperature, moisture content of materials, cycles of extraction could affect the extraction efficiency of analytical methods for determination chemical compounds. In current study, two effects of relatively crucial factors (extraction methods and choice of the solvent), were investigated. Generally, hexane and chloroform which was used as a non-polar solvent have achieved high recoveries in MA method, the responses of the non-polar compounds were significantly improved, Figures 1&2. On the other hand, ethylacetate and butanol which was used as a polar solvent have achieved high recoveries in SX method, the responses of the polar compounds were significantly improved, Figures 3&4. This is due to enhance the polarity of the solvent and consequently can decrease the adsorption of polar constituents onto soil.

Extraction and fractionation

Powdered of air-dried aerial parts of the plant (1.0 kg) will be divided into two portions, 500 gm for each; extracted by absolute alcohol at room temperature until exhaustion by using two different extraction methods maceration (MA) and SX (SX) apparatus. Both extracts were filtered and concentrated under reduced pressure, using rotary evaporator at 40°C. Both portions dried extracts were suspended in water and successively fractionated with hexane (HMA, HSX), chloroform (CMA, CSX), ethyl acetate (EMA, ESX) and n-butanol

(BMA, BSX) saturated with water. The resulting fractions are going to be concentrated to dryness using rotary evaporator, weighed and subjected to GC/MS studies. A preliminary phytochemical analysis of the plant fractions was carried out by using TLC plates with different solvents system. The chromatogram was air dried and visualized using short and long wave length as well as different chemical reagents to detect the presence of compounds. The resulting fractions are subjected to analyzed by GC & GC/MS.

GC/MS

An Agilent 7890 gas chromatograph (GC) was coupled to a 240-series ion trap mass spectrometer (MS) detector available at Central Laboratory, College of Pharmacy, King Saud University, Riyadh. An aliquot of 2 µL of extract was injected into the GC column Elite -5MS of 30 m long, 0.25 µm film thickness, 0.25 mm internal diameters.

Chromatographic Analysis of compounds

The system was equipped with a DB-5MS capillary column (30 m, 0.25-mm internal diameter, 0.25-mm film). Carrier gas was Helium used at a constant flow (1.5 ml/min). Two microliters of the extracts were injected in splitless mode, and the injector temperature was 250°C. The initial temperature was 100°C (hold 2.0 min) then the oven temperature was programmed from 100°C to 150°C (hold 1.0 min) at a rate of 5 °C/min, then from 150°C to 200°C (hold 2.0 min) at a rate of 5 °C/min, next from 200°C to 240°C (hold 2.0 min) at a rate of 10 °C/min, finally from 240°C to 260°C (hold 12 min) at a rate of 10 °C/min. The Equilibration time was 3 min. Mass spectral detection was carried out in electron ionization mode by scanning at 40 to 600 a.m.u. Finally, unknown compounds were identified by comparing the spectra with that of the National Institute of Standard and Technology library (NIST 2005) and Wiley Library 2006 (Ver 2.1). The whole time needed for analyzing a single sample was 61 minutes. The Retention Index (RI) was assessed by running the standard solution of C-7 to C-30 saturated alkanes standard from SUPELCO with the same method as sample. The concentration of alkanes was 1000 µg/ml. The RI values was assessed by AMDIS software 32.

RESULTS AND DISCUSSION

The components were identified based on GC retention time and matching with Wiley 2006 library as well as by comparison the fragmentation patterns of their mass spectra with those reported in the literatures **(Adams, 1995; Mclafferty, and Staffer, 1989)** and various of the identified components were identified as sterols, fatty acids, alkanes and alcohols compounds. Analysis of the two hexane extracts of *T. nubica*, **Figure 1**, resulted in eighteen and six components representing 88.72% & 76.31% for HMA and HSX respectively, their retention indices and area percentages (concentrations) are summarized in Tables 1&5. The major compounds of HMA and HSX were hexadecenoic acid (**6**, 16.3%), hexatriacontane (**18**, 15.9%), cholest-5-en-3-ol (3 Beta) (**13**, 9.83%), bicyclo [2.2.1] heptane (**1**, 9.01%), and 2-pentadecanone (**5**, 8.53%) as well as hexadecenoic acid (**2**, 45.42%), 1,2-benzenedicarboxylic acid (**6**, 10.72%) and octadecanoic acid (**4**, 9.7%) respectively. However, the minor components of these two different extracts were dihydromyrcenol (**2**, 0.44%), 5-undecen-2-one (**3**, 1.16%), and elcosyl acetate (**7**, 1.18%) as well as 2-octyldodecan-1-ol (**3**, 1.91%), and 2-propyldecan-1-ol (**5**, 2.77%) respectively, Tables 1&5. By comparison between HMA & HSX extracts, it was found that the major compound of the two different extracts was hexadecenoic acid (**6**, 16.3%) and (**2**, 45.42%) respectively with higher percentage by three times in HSX, there are considerable variation in the amount of the major component obtained from different extraction methods. On the other hand, it was found that the results are completely different as the rest components of the HMA and HSX.

Also, analysis of the two chloroform extracts of *T. nubica*, **Figure 2**, resulted in eighteen and ten components representing 99.15% & 92.24% for CMA and CSX respectively, their retention indices and area percentages (concentrations) are summarized in Tables 2&6. The major compounds of CMA and CSX were bicyclo [2.2.1] heptane-exo-2 (6, 40.38%), 1,2,3-propanetriol (2, 22.57%), and 3,7-decadien-2-one (7, 6.65%) as well as beta-D-fructopyranose (2, 27.13%), 2-undecanone (5, 15.63%), methyl alpha-ketopalmitate (10, 11.44%), hexadecenoic acid (8, 10.77%) and cholesta-5,7,9 (11)-trien-3-ol (4, 8.07%). However, the minor components of these two different extracts were methyl 3-trans-2,3-epoxybutane (**1**, 0.37%), 9-octadecenoic acid (**17**, 0.49%), and globulol (**5**, 4.57%) as well as 2,3-anhydro-D-galactosan (**7**, 1.91%), octadecanal (6, 2.67%) and 2-propyldecan-1-ol (**9**, 2.77%) respectively, Tables 2&6. By comparison between CMA & CSX extracts, it was noticed that completely different in the composition and number of compounds by two different extract methods.

Furthermore, analysis of the two ethyl acetate extracts of *T. nubica*, **Figure 3**, resulted in ten and fourteen components representing 94.75% & 96.15% for EMA and ESX respectively, their retention indices and area percentages (concentrations) are summarized in Tables 3&7. The major compounds of EMA and ESX were tetracosane (**7**, 39.27%), bicyclo [2.2.1] heptane-exo-2 (**1**, 20.35%), and 1,2-benzenedicarboxylic acid (**9**, 14.61%), as well as mome inositol (**4**, 24.21%), hexadecanoic acid (**6**, 20.73%) and megastigma-3,7 €, 9-triene (**2**, 13.53%) respectively. However, the minor components of these two different extracts were octadecanal (**10**, 1.62%), decanoic acid (**5**, 1.99%), and bis-(3,5,5-trimethylhexyl) ether (**6**, 2.15%) as well as methyl alpha-ketopalmitate (**8**, 1.61%), and 2-propyldecan-1-ol (**9**, 1.8%) respectively, Tables 3&7. By comparison between EMA & ESX extracts, it was

noticed that completely different in the composition and number of compounds by two different extract methods.

Analysis of the two butanol extracts of *T. nubica*, **Figure 4**, resulted in seventeen components representing 99.41% & 95.59% for BMA and BSX respectively, their retention indices and area percentages (concentrations) are summarized in Tables 4&8. The major compounds of BMA and BSX were mome inositol (5, 77.47%), and bicyclo [2.2.1] heptane-exo-2 (**3**, 4.34%), as well as hexadecenoic acid (**12**, 38.18%), and 2-pentadecanone (**7**, 7.49%) respectively. However, the minor components of these two different extracts were Dl-3,4-dimethyl-3,4-hexanediol (**7**, 0.51%), eicosyl acetate (**10**, 0.15%), and methyl ester of octanoic acid (**8**, 0.30%) as well as oxalic acid (**9**, 0.31%), 2,3-epoxyhexanol (5, 0.46%), and 6,10-dimethyl-4-undecanol (**14**, 0.82%) respectively, Tables 4&8. By comparison between BMA & BSX extracts, it was observed that completely different in the composition of compounds by two different extract methods.

This is the first time to study *T. nubica* species growing in Saudi Arabia. There are no previous GC &GC/MS studies on all *Tephrosia* species to compare our results with the reported studies for the same plant. From our data, we observed that more number of compounds extracted from HMA & CMA than HSX & CSX, however, less number of compounds have been shown in EMA & BMA than ESX & BSX; this might be due to the compounds in both HMA & CMA extraction are heat labile which might be decomposed when exposure to heat. On the other hand, the conventional SX has given a high recovery of the compounds from ethyl acetate and butanol extractions than in Maceration methods; this might be due to stability of compounds in these two extractions and need heat to be extracted efficiently. So, it could be noticed that the extraction method affects quantitatively and qualitatively on the chemical compounds of the all extracts of *T. nubica*. This observed variation might be owing to different experimental condition that using cool and heat extractions by maceration and SX experiment respectively.

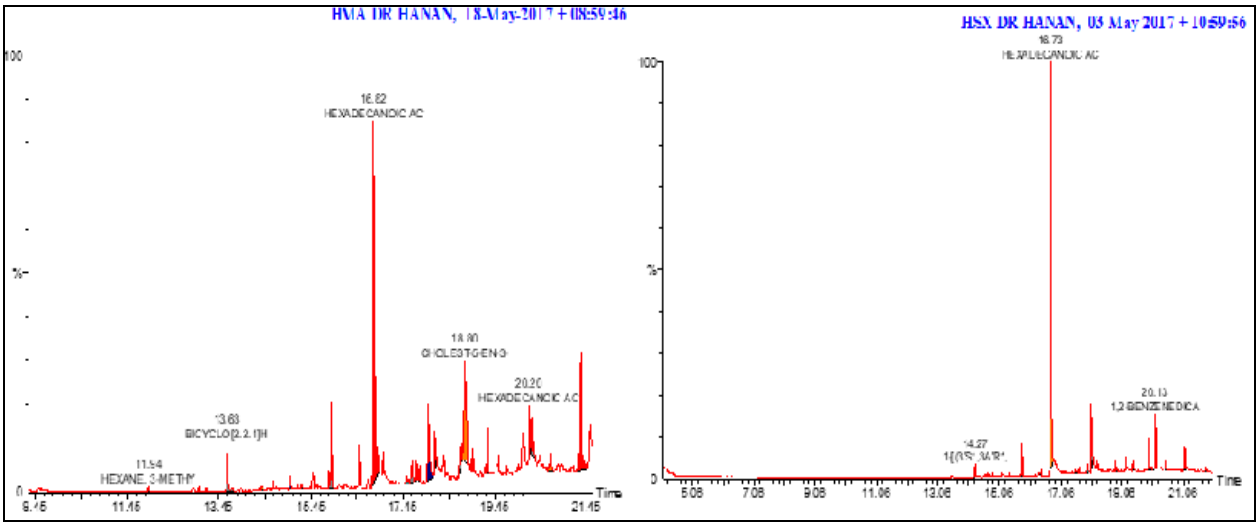

Figure 1. GC/MS chromatogram of the two Hexane extracts; (HMA) Macerated and Soxhlet (HSX) of *T.nubica* grown in SA.

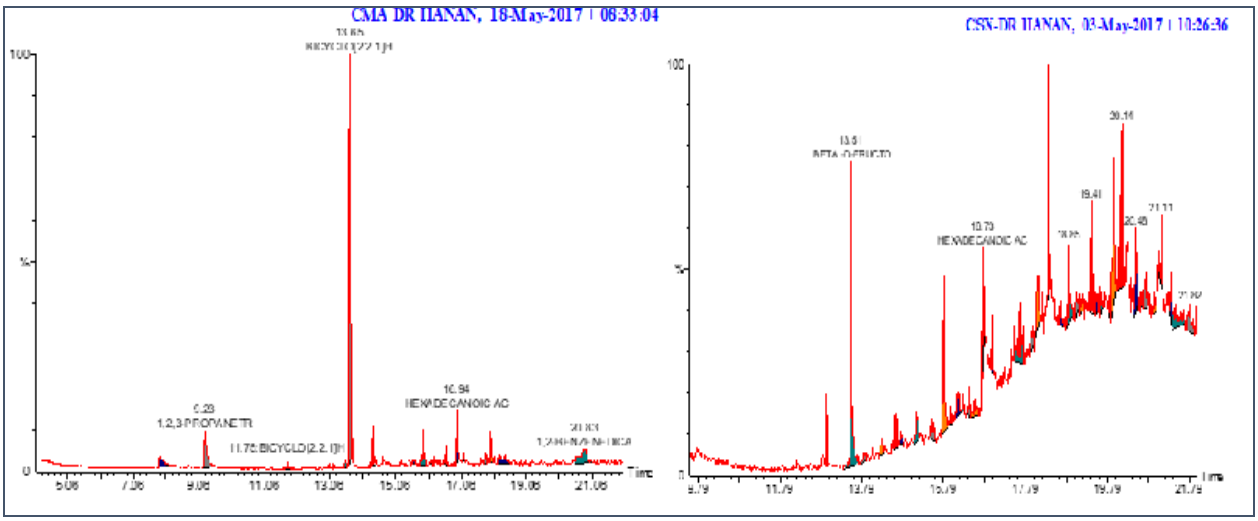

Figure 2. GC/MS chromatogram of the two Chloroform extracts; (CMA)Macerated and Soxhlet (CSX) of *T.nubica* grown in SA.

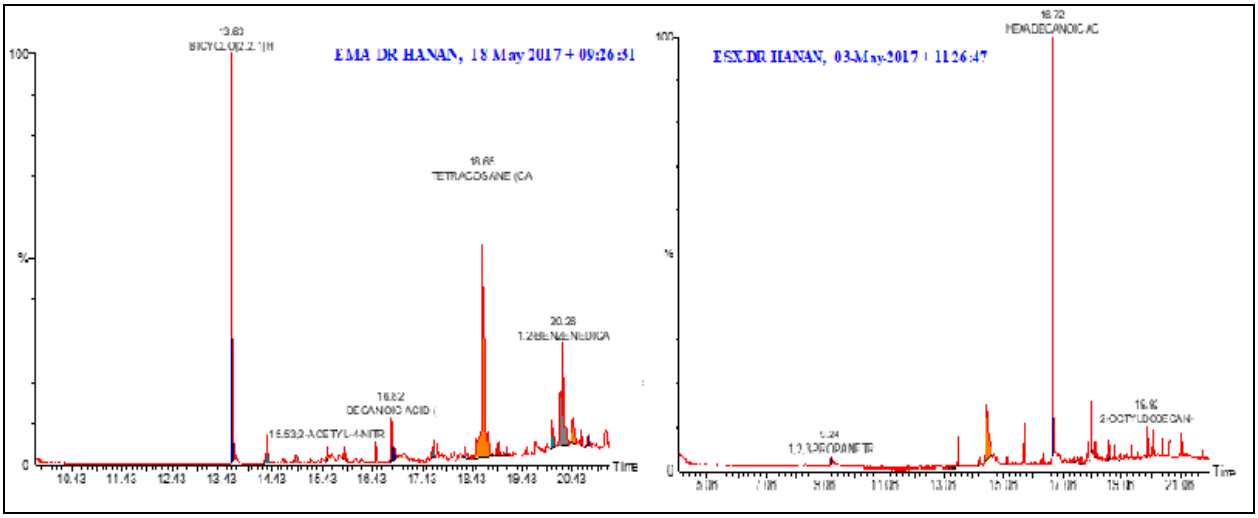

Figure 3. GC/MS chromatogram of the two Ethyl acetate extracts; (EMA)Macerated and Soxhlet (ESX) of *T.nubica* grown in SA.

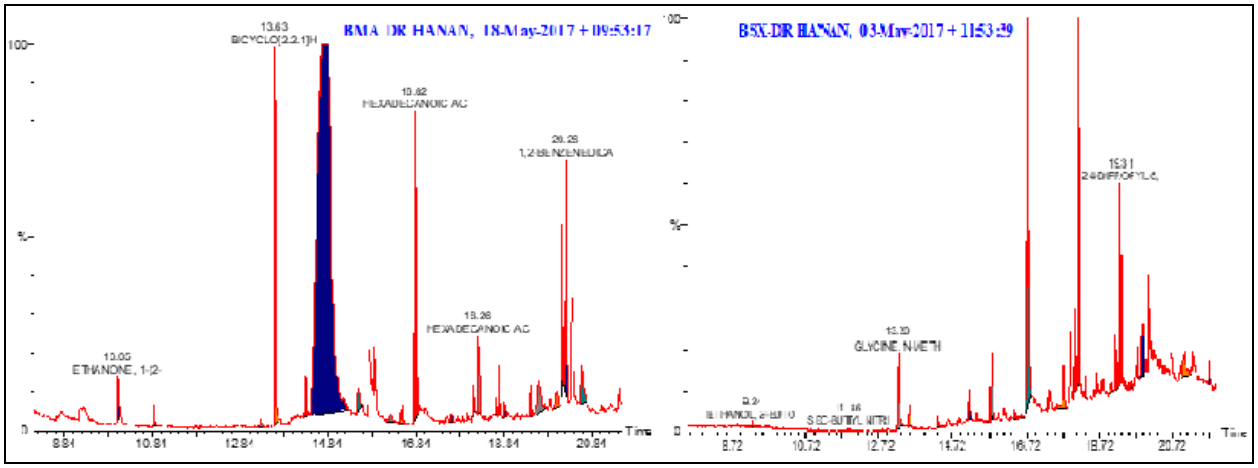

Figure 4. GC/MS chromatogram of the two Butanol extracts; (BMA)Macerated and Soxhlet (BSX) of *T. nubica* grown in SA.

Table 1. Chemical composition of the Hexane Macerated extract (HMA) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area %	Area	RI
1	BICYCLO[2.2.1]HEPTANE	13.63	9.010	55.150	24733	1606
2	DIHYDROMYRCENOL	14.38	0.430	2.610	1171	1684
3	5-UNDECEN-2-ONE	14.62	1.160	7.080	3173	1723
4	PALUSTROL	15.02	2.760	16.900	7580	1756
5	2-PENTADECANONE	15.89	8.530	52.200	23408	1851
6	HEXADECANOIC ACID	16.82	16.330	100.000	44847	1971
7	EICOSYL ACETATE	17.56	1.180	7.220	3236	1996
8	BIS-(3,5,5-TRIMETHYLHEXYL) ETHER	17.66	1.710	10.480	4699	2016
9	METHYL ALPHA-KETOPALMITATE	17.75	2.520	15.410	6909	2065
10	2-PIPERIDINONE	17.82	1.080	6.620	2971	2085
11	9-OCTADECENOIC ACID	18.16	2.090	12.800	5741	2138
12	CHOLESTEROL ISOCAPROATE	18.71	3.190	19.530	8757	2236
13	CHOLEST-5-EN-3-OL (3.BETA.)	18.80	9.830	60.200	26996	2251
14	2-PROPYLDECAN-1-OL	18.96	2.070	12.660	5680	2273
15	2-HEXADECENE	19.29	5.340	32.690	14660	2319
16	HEXADECANOIC ACID	20.20	2.970	18.200	8162	2447
17	DOCOSANE	20.66	2.620	16.070	7206	2473
18	HEXATRIACONTANE	21.32	15.900	97.350	43659	2605

Table 2. Chemical composition of the Chloroform Macerated extract (CMA) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area	%Area	RI
1	METHYL 3-TRANS-2,3-EPOXYBUTANE	8.71	0.370	0.920	4636	1156
2	1,2,3-PROPANETRIOL	9.23	22.570	55.900	280642	1262
3	BICYCLO [2.2.1] HEPTANE-EXO-2	11.75	2.660	6.590	33090	1457
4	10-METHYL-8-TETRADECEN-1-OL	13.01	0.800	1.990	9969	1512
5	GLOBULOL	13.19	0.510	1.250	6288	1584
6	BICYCLO [2.2.1] HEPTANE-EXO-2	13.65	40.380	100.000	502072	1608
7	3,7-DECADIEN-2-ONE	14.35	6.650	16.470	82703	1677
8	1,2:8,9-DIEPOXY-P-MENTHANE-7-YL	15.61	1.940	4.800	24094	1695
9	NEOPHYTADIENE	15.91	1.360	3.380	16950	1852
10	EICOS-2-YNE	16.26	0.510	1.260	6325	1894
11	HEPTADECANOIC ACID	16.61	3.200	7.920	39780	1943
12	HEXADECANOIC ACID	16.94	4.830	11.970	60096	1988
13	NONADECANOIC ACID	17.12	1.010	2.510	12607	2012
14	1-OCTADECANOL	17.80	1.430	3.550	17826	2108
15	2-HEXADECEN-1-OL	17.97	5.330	13.210	66323	2132
16	TETRADECANOIC ACID	18.08	1.200	2.970	14930	2143
17	9-OCTADECENOIC ACID	18.39	0.490	1.220	6102	2234
18	1,2-BENZENEDICARBOXYLIC ACID	20.82	3.910	9.700	48676	2536

Table 3. Chemical composition of the Ethyl Acetate Macerated extract (EMA) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area %	Area	RI
1	BICYCLO[2.2.1]HEPTANE-EXO-2	13.63	20.350	51.840	48767	1605
2	1,3-DIOXOLANE-2-PENTANOIC ACID	14.33	5.260	13.390	12600	1674
3	2,7-OCTANEDIONE	14.90	4.560	11.610	10925	1712
4	2-ACETYL-4-NITROCYCLONONANONE	15.53	2.740	6.980	6567	1806
5	DECANOIC ACID	16.82	1.990	5.060	4759	1971
6	BIS-(3,5,5-TRIMETHYLHEXYL) ETHER	17.66	2.150	5.480	5159	2087
7	TETRACOSANE	18.65	39.270	100.000	94078	2227
8	3-BUTENE-2-OL	20.05	2.200	5.590	5263	2426
9	1,2-BENZENEDICARBOXYLIC ACID	20.26	14.610	37.210	35003	2455
10	OCTADECANAL	20.76	1.620	4.140	3891	2661

Table 4. Chemical composition of the Butanol Macerated extract (BMA) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area%	Area	RI
1	1-(2-HYDROXY-5-METHYL)-ETHANONE	10.05	1.150	1.480	17813	1329
2	DIBUTYL ESTER	10.89	1.340	1.720	20688	1562
3	BICYCLO[2.2.1]HEPTANE-EXO-2	13.63	4.340	5.610	67289	1606
4	1-(3-BUTYLOXIRANYL)-ETHANONE	14.35	1.720	2.220	26645	1676
5	MOME INOSITOL	14.82	77.470	100.000	1200333	1726
6	1,3-DIOXOLANE-2-PROPANAL	15.54	0.930	1.210	14472	1735
7	DL-3,4-DIMETHYL-3,4-HEXANEDIOL	16.26	0.150	0.190	2281	1851
8	METHYL ESTER OF OCTANOIC ACID	16.52	0.300	0.390	4713	1925
9	HEXADECANOIC ACID	16.82	2.430	3.140	37682	1970
10	EICOSYL ACETATE	17.64	0.150	0.190	2339	2051
11	METHYL ALPHA-KETOPALMITATE	18.74	0.550	0.720	8583	2215
12	3,5,5-TRIMETHYLHEXYL -2-(DIMETHYL	18.86	0.600	0.770	9286	2262
13	2,4-DIPROPYL-5,5-DIMETHYL-1,3- OCTANE	19.44	0.640	0.830	9925	2325
14	DOCOSANE	19.65	2.730	3.520	42261	2370
15	HEXANOIC ACID	20.05	0.490	0.630	7541	2412
16	1,2-BENZENEDICARBOXYLIC ACID	20.26	2.960	3.820	45854	2455
17	OCTADECANAL	20.62	1.460	1.880	22579	2507

Table 5. Chemical composition of the Hexane Soxhlet extract (HSX) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area %	Area	RI
1	2-PENTADECANONE	15.81	5.800	12.780	18563	1183
2	HEXADECANOIC ACID	16.73	45.420	100.000	145252	1945
3	2-OCTYLDODECAN-1-OL	17.64	1.910	4.210	6117	2405
4	OCTADECANOIC ACID	18.05	9.700	21.350	31009	2439
5	2-PROPYLDECAN-1-OL	18.83	2.770	6.100	8856	2576
6	1,2-BENZENEDICARBOXYLIC ACID	20.13	10.720	23.610	34287	2589

Table 6. Chemical composition of the Chloroform Soxhlet extract (CSX) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area %	Area	RI
1	CARVONE OXIDE	12.93	5.890	21.710	3510	1195
2	BETA.-D-FRUCTOPYRANOSE	13.51	27.130	100.000	16169	1595
3	1-(METHYLENCYCLOPROPYL)-ETHANOL	14.27	4.030	14.850	2401	
4	CHOLESTA-5,7,9(11)-TRIEN-3-OL	15.12	8.070	29.750	4810	1785
5	2-UNDECANONE	15.81	15.630	57.610	9315	1840
6	OCTADECANAL	16.41	2.670	9.850	1592	2145
7	2,3-ANHYDRO-D-GALACTOSAN	16.54	1.910	7.050	1140	1854
8	HEXADECANOIC ACID	16.73	10.870	40.070	6479	1942
9	2-PROPYLDECAN-1-OL	16.97	4.570	16.850	2725	2176
10	METHYL ALPHA-KETOPALMITATE	18.10	11.440	42.190	6821	2195

Table 7. Chemical composition of the Ethyl Acetate Soxhlet extract (ESX) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area %	Area	RI
1	1,2,3-PROPANETRIOL	9.24	8.310	34.300	43809	1263
2	MEGASTIGMA-3,7(E),9-TRIENE	13.57	13.530	55.890	71388	1599
3	2-BUTANOL	14.27	3.580	14.810	18910	1650
4	MOME INOSITOL	14.51	24.210	100.000	127724	1692
5	4-HEPTANOL	15.14	2.050	8.470	10821	1785
6	HEXADECANOIC ACID	16.72	20.730	85.610	109347	1958
7	OCTADECANOIC ACID	18.04	5.360	22.140	28276	2143
8	METHYL ALPHA-KETOPALMITATE	18.64	1.610	6.640	8476	2184
9	2-PROPYLDECAN-1-OL	18.83	1.800	7.420	9477	2196
10	2-OCTYLDODECAN-1-OL	19.92	3.180	13.120	16758	2409
11	9-OCTADECENOIC ACID	20.12	2.900	11.970	15285	2436
12	OCTADECANAL	20.12	2.900	11.970	15285	2438
13	2-OCTYLDODECAN-1-OL	20.47	2.410	9.960	12718	2485
14	2-PROPYLDECAN-1-OL	21.08	3.580	14.790	18887	2572

Table 8. Chemical composition of the Butanol Soxhlet extract (BSX) of *T. nubica* grown in SA.

#	Name	RT	Area %	N Area %	Area	RI
1	GLYCINE	13.20	5.630	14.730	21274	1569
2	BETA.-D-FRUCTOPYRANOSE	13.51	3.340	8.760	12641	1595
3	2-PROPANONE	14.31	2.220	5.810	8388	1626
4	NONANOIC ACID	15.17	2.170	5.690	8220	1764
5	2,3-EPOXYHEXANOL	15.66	0.460	1.220	1757	1812
6	NEOPHYTADIENE	15.75	2.040	5.340	7712	1834
7	2-PENTADECANONE	15.80	7.490	19.610	28317	1840
8	TETRADECANOIC ACID	16.43	1.480	3.880	5601	1957
9	OXALIC ACID	16.58	0.310	0.810	1164	1985
10	HEXADECANOIC ACID	16.72	16.620	43.530	62850	1963
11	2-PROPYLDECAN-1-OL	17.70	1.490	3.890	5622	2095
12	HEXADECANOIC ACID	18.16	38.180	100.000	144381	2158
13	2-PROPYLDECAN-1-OL	18.25	3.530	9.250	13351	2195
14	6,10-DIMETHYL-4-UNDECANOL	18.31	0.820	2.150	3107	2231
15	TETRACONTANE	19.15	2.370	6.210	8972	2299
16	1-TETRADECENE	20.04	3.210	8.400	12123	2424
17	HEXANOIC ACID	21.08	4.140	10.840	15648	2526

CONCLUSION

This work presents information for ensuring the existing extraction method is achievable in the searching and teaching laboratory, also permitting students hands-on investigative experience. Also, providing and supporting scientific thinking, laboratory skills, implemented and competency, this lab experiment allows students to partake in an interpretation with several discipline approaches **(Luque de Castro et al., 1998**). The qualitative and quantitative analysis of *T. nubica* by using GC/MS have permitted to separate and identify chemical constituents of 18, 18, 10, 17 for HMA, CMA, EMA and BMA, as well as 6, 10, 14 and 17 for HSX, CSX, ESX and BSX respectively. We noticed that the MA method can be improved for giving satisfactory results and can be selected for being a good and fast alternative of the classical technique, SX in non-polar and heat labile compounds. however, we also noticed that the SX method can be improved for giving reliable results and could be selected for being a good and fast alternative of the classical technique, MA in polar and heat stable compounds. To improve the performance of an extraction methods, it is important to consider the cost, the amount of solvent consumption, the extraction time and the ease of handling such techniques. We need further biological application on these compounds which were identified by two extraction methods and we look for different biological response.

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