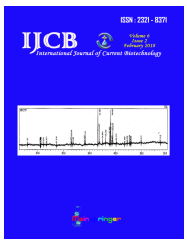
International Journal of Current Biotechnology
ISSN: 2321 - 8371
Journal Homepage : http://ijcb.mainspringer.com



Analysis of bioactive components in ethanolic extract of Cashew Nut Shell Oil using HPTLC and GC-MS techniques

Dorathy Selva Jeba Pritha S[1*] and Karpagam S[2]

[1]Research Scholar, Department of Botany, Queen Mary's College, Chennai -600 004.
[2]Associate Professor, Department of Botany, Queen Mary's College, Chennai-600 004.

ARTICLE INFO

Article History:
Received 04 December 2017
Received in revised form 25 January 2018
Accepted 15 February 2018
Available online 28 February 2018

Key words:
Cashew Nut Shell Oil, HPTLC, GC-MS.

ABSTRACT

The extraction of phytochemicals from the plant materials has been reported to have tremendous research interest and proved great potential. This work highlights the importance of Cashew Nut Shell Oil (CNSO) and determines the bioactive compounds. The separation of bioactive constituents done by HPTLC method on silica gel precoated aluminium plates by using solvent system :Toluene : ethyl acetate : glacial acetic acid (9:1:0.2). Scanning of HPTLC fingerprint at 254nm, 366nm and visible light. The bands showed the presence of green, blue, violet and brown revealed the presence of steroids, terpenoids and saponins after spraying with Vanillin- sulphuric acid. In GC-MS analysis different peaks with high and low molecular weight were determined and identified the presence of thirty bioactive compounds. The results of the present study explore the presence of potential phytocomponents that serve in qualitative, quantitative analysis and appropriate for standardization of the extract for therapeutic properties.

Introduction

The Cashew tree, *Anacardium occidentale* is native to Eastern Brazil. The Portuguese , in 16th and 17th centuries introduced this species into the tropical regions like India, Africa, Indonesia and South East Asia.(Marcionilia *et al*., 2009). In Brazil the Cashew apple is used to prepare jams and soft alcoholic drinks (De lima,*et al*., 2008). The ethanolic extract of Coconut shell oil contains phytochemicals which is used in treatment of wounds. (Dorathy and karpagam, 2017, a). A variety of products based on CNSO are used as anti-oxidants, stabilizers and emulsifiers for the petroleum products (Menon,*et al*., 1985). Besides commercial values it is also produced for its biological properties like Antitumour (Wu,Y,*et al*., 2011) and antioxidant activities (Oliveria, *et al*., 2010; Trevisan, *et al*., 2006). Antibacterial activity of CNSO is high against *Staphylococcus aureus, E.coli, Enterococcus* and *Salmonella* (Dorathy and karpagam., 2017, b). Anacardic acid with methicillin is resistant to *Staphylococcus aureus* (Parasal, *et al*., 2011). The higher concentration of cardanol 86% with phenols 5% isused in resins (Setianto, *et al*., 2001). Lower quantities of Hydroxyl alkyl phenols were obtained from Cashewnut shell (Gomez, *et al*.,2010). Unsaturation of the side chain of anacardic acid increases its action against free radicals, AChE enzyme and *Artemia salina* nauplii (Selene M Morais, *et al*.,2017).

*Corresponding author.
Email address: cdorathypritha@gmail.com

Materials and Methods

Plant materials

The cashew nuts were collected from cashew plantation of Panruti, Cuddalore District, Tamilnadu, India. The seed coat of cashew nut were separated, dried till the removal of moisture content and ground into fine powder. Grounded cashew nut shells (250g) were heated in a earthern pot for a span of three hours giving a yield of approximately 25 cc of oil.

HPTLC Analysis: High Performance Thin Layer Chromatographic (HPTLC) studies were carried out as per the procedures described by Wagner and Bladts, (1996). The ethanol extract of CNSO was used as sample and applied onto the plates with automated CAMAG HPTLC system that programmed through WIN CATS software. After the application of the spots, the plates were developed in twin trough glass chamber. A TLC scanner with win CATS software was used for scanning the TLC plates. The samples (0.5µl) were applied in TLC aluminium silica gel $60F_{254}$ (E.Merck)

The mobile phase consisted of Toluene: Ethyl acetate: Glacial Acetic acid (9:1:0.2) (v/v). 15ml of mobile phase was utilized per plate. After development the plate was allowed to dry in air and scanned under remission mode at UV-254nm, UV-366nm and visible light by densitometric scanner after derivatised using vanillin – sulphuric (VS)

acid. The spots and peaks were detected and their Rf values and peak areas were recorded.

2.3 GCMS (Gas chromatography – Mass Spectrometry) Analysis

The phytochemical search of ethanolic extract was performed on GC Clarus 500 Perkin Elmer system interfaced to a mass spectrometer. The software used is Turbomass ver 5.2 column Elite 5ms was fused with the silica capillary column (30 x 0.25 mm ID x 0.25µm film thickness, 5%Phenyl, 95%Dimethyl Polysiloxane). Electron impact mode operated at 70 eV., Helium gas (99.999%) was used as the carrier gas at 1ml/ min of constant flow rate with injector temperature of 290°C. Electron ionization occurs and the ion source temperature was 150°C. In the gas chromatography the temperature program was as follows from 50°C raised to 220°C at 2°C/ min hold for 10min; From 220°C to 280°C with 4°C/min hold for 10 min. Identification of unknown compounds were done by referring the retention times with authentic compounds and the spectral data collected from NIST and Wiley Spectral library search programme.

Results and Discussions

Many bioactive compounds were present in the ethanolic extract of CNSO. The images of HPTLC clearly indicate that all the sample constituents were separated without any tailing and diffuseness shown in Fig.1. The HPTLC fingerprint scanned at 254nm revealed the presence of 11 polyvalent phytoconstituents depicted in Table.1 TLC plate showed different colour phytoconstituents of CNSO as green, blue, violet, brown, grey and pink bands showing the presence of terpenoids, steroids and saponins after spraying with Vanillin sulphuric acid reagent.

The Rf values ranged from 0.03 to 0.97 and area ranged from 212.5 to 18199.5AU as shown in Table.2 and Fig.2. Out of 14 peaks, the components with Rf values 0.63 and 0.53were predominant area 15126.5 and 18199.8 respectively. The densitometric results of CNSO at 366nm exhibited 10 peaks with Rf values ranging from 0.06 to 0.96 as illustrated in Fig.3. The area was ranging from 130.9 to 7412.2AU as shown in Table.3.

Identification of various secondary metabolites present in the plant CNSO can be confirmed by GC-MS with different retention times as illustrated in Fig.4 (Al-Huqail et al, 2015, Payum., 2016). The relative percentage amount of each compound can be calculated by its average peak area with the total areas. National Institute of Standard and Technology (NIST) database were used in interpretation of GC-MS of separated components. In the ethanolic extract of CNSO 30 bioactive compounds were detected. The peak names, their molecular formula, molecular weight, retention time and peak areas are summarized in Table.4. Further research in this extract leads to isolation of biomolecules and their structural elucidation will be useful for drug development.

Conclusion

HPTLC analysis of the plant extract gives the adequate information about the parameters for the quality of herbal formulations. The results obtained from the HPTLC fingerprin and GCMS analysis on the ethanolic extract of CNSO provides qualitative evaluation of the drug and therapeutic potency. Further isolation of the compounds and determination of their specific activity can be made for the utilization of compounds in pharmacological efficacy.

References

Al-Huqail, Asma A, Elgally GA, Ibrahim MM (2015). Identification of bioactive phytochemical from two *Punica* species using GC-MS and estimation of antioxidant activity of seed extracts. Saudi journal of Biological Sciences. http://dx.doi.org/10.1016/j.sjbs. 2015.11.009.

De Lima S G., Feitosa C M., Cito A M G L., et al., (2008). Effects of immature Cashew nut shell liquid (Anacardium occidentale) against oxidative damage in *Saccharomyces cerevisiae* and inhibition of acetyl choline sterease activity. Journal of Genetics and Molecular Research, 7 (3): 806-818.

Dorathy Selva Jeba Pritha, S.Karpagam, (2017. a). Evaluation of phytochemical content of Coconut Shell oil. National Journal of Advanced Research, Vol.3; issue 2:1-2.

Dorathy Selva Jeba Pritha, S.Karpagam, (2017. b). Phytochemical content and Antimicrobial activity of Cashew Nut Shell Oil. IOSR Journal of Pharmacy and Biological Sciences. Vol.12(4)61-64.

Gomez-Caravoca AM, Veradov Caboni MF (2010). Chromatographic techniques for the determination of alkyl phenols, tocopherols andother minor polar compounds in raw and roasted cold pressed Cashewnut oils. J.Chromatogr A Nov 19; 1217 (47):7411-7417.

Marcionilia FP, Denis D L, Leonardo R M.,Adilson B, Sandra T S and Leticia V C L., (2009). Ecotoxicological analysis of cashew nut industry effluents , specifically two of its major phenolic components, cardol and cardanol. Pan American Journal of Aquatic Sciences, 4 (3): 363-368.

Menon A R R, Pillai c K S and Mathew A G, (1985). Cashew nut shell liquid- its polymeric and other industrial products, Journal of Science in Industrial Research, 44: 324-338.

Morais TC, Pinto NB, Carvalho KM, Rios JB, Ricardo NM, Trevisan MT, Rao VS, Santos FA (2010). Protective effect of anacardic acids from Cashew (*Anacardium occidentale*) on ethanol induced gastric damage in mice.Chem. Biol. Interact 183: 264-269.

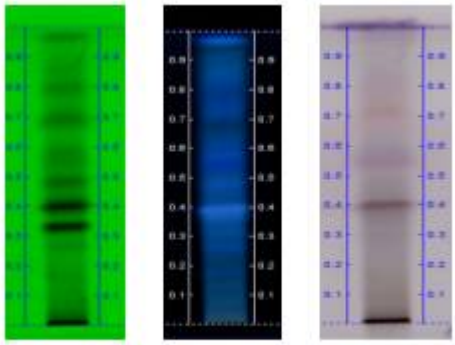
Oliveira MSC, Morais S M, Maglhaes DV, Batista WP,Vieira IGP, Craveiro AA, Menezes JESA, Carvallo AFU, Lima GPU (2010). Antioxidant, Larvicidal and Antioxidant, Larvicidal and Antiacetylcholinesterase activities of Cashew nut shell liquid consitutents. Acta. Trop. 117: 165-170.

Parasal LS,Srinivasa Rao Tumati, L Cyril, Arun Kumar, Srinivasa Prasad chigurupati and G.S.Rao (2011) Invitro Antimicrobial Activity of Cashew (*Anacardium occidentale*) Nuts shell liquid Against methicillin Resistant *Staphyloccus aureus* (MRSA) clinical isolates , Int. J.Pharm Sci,Vol3(4):436-440.

Payum T(2016). GC-MS Analysis of *Mussaenda roxburghii* Hk.f.A Folk Food plant used among Tribes of Arunachel Pradesh, India. Pharmacognosy Journal, 8(4):395-8.

Selene MMorais,Katherine A.Silva, Halisson Araujo, Icaro G P Viera, Daniela R Alves, Raquel OS Fontenlle and Artur MS Silva, (2017). Anacardic acid constituents from Cashew nut shell liquid: NMR characterization and

Figure – 1: Photo documentation under UV and VS reagent



UV 254nm UV 366nm VS reagent

Figure – 2: HPTLC finger print of CNSO at 254nm

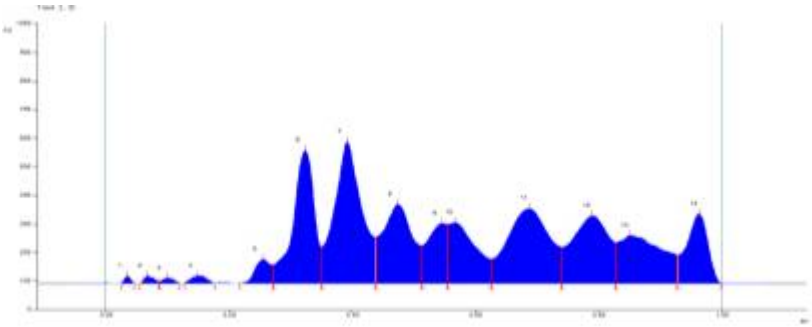


Figure – 3: HPTLC finger print of CNSO at 366 nm

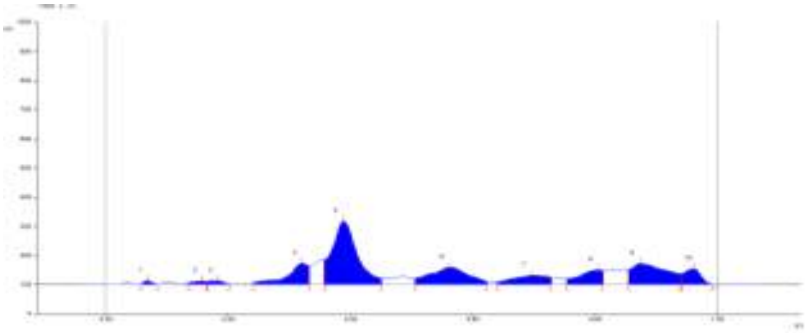


Figure – 4: GCMS Chromatogram of ethanolic extract of CNSO

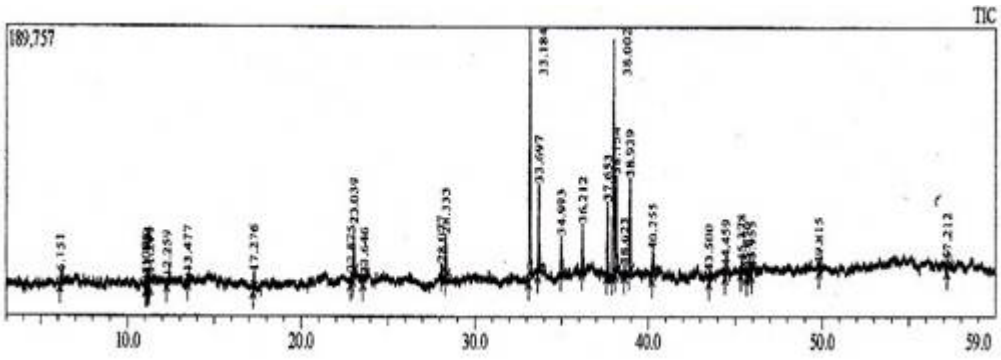


Table – 1 : HPTLC data of CNSO at UV 254nm, 366nm and VS reagent

Solvent system	Rf values		
	UV 254nm	UV 366nm	VS reagent
Toluene : Ethyl acetate : Glacial acetic acid (9:1:0.2)	0.97 Green	0.95 Blue	0.80 Light violet
	0.85 Green	0.85 Violet	0.70 Light brown
	0.79 Green	0.71 Violet	0.55 Light grey
	0.68 Green	0.57 Blue	0.53 Light pink
	0.57 Green	0.55 Violet	0.40 Dark brown
	0.50 Green	0.49 Violet	
	0.48 Green	0.40 Brown	
	0.40 Dark green	0.31 Violet	
	0.35 Dark green	0.25 Violet	
	0.26 Green	0.14 Light violet	
	0.15 green	0.12 light violet	

Table – 2: Rf value of CNSO at 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.3 AU	0.04 Rf	25.1 AU	0.92 %	0.05 Rf	0.5 AU	212.5 AU	0.21 %
2	0.06 Rf	0.1 AU	0.07 Rf	25.4 AU	0.93 %	0.09 Rf	7.4 AU	345.9 AU	0.34 %
3	0.09 Rf	7.5 AU	0.10 Rf	18.4 AU	0.67 %	0.12 Rf	0.5 AU	273.1 AU	0.27 %
4	0.13 Rf	0.7 AU	0.15 Rf	25.5 AU	0.93 %	0.18 Rf	0.5 AU	554.5 AU	0.54 %
5	0.22 Rf	0.6 AU	0.26 Rf	82.9 AU	3.02 %	0.27 Rf	64.8 AU	1778.4 AU	1.73 %
6	0.27 Rf	65.4 AU	0.32 Rf	464.5 AU	16.95 %	0.35 Rf	27.1 AU	13384.4 AU	13.03 %
7	0.35 Rf	128.1 AU	0.39 Rf	494.8 AU	18.06 %	0.44 Rf	61.7 AU	18199.8 AU	17.72 %
8	0.44 Rf	161.8 AU	0.47 Rf	275.8 AU	10.07 %	0.51 Rf	31.8 AU	10999.5 AU	10.71 %
9	0.52 Rf	132.5 AU	0.55 Rf	208.7 AU	7.62 %	0.56 Rf	07.0 AU	5414.9 AU	5.27 %
10	0.56 Rf	206.7 AU	0.57 Rf	212.0 AU	7.74 %	0.63 Rf	84.7 AU	7898.4 AU	7.69 %
11	0.63 Rf	85.3 AU	0.69 Rf	261.4 AU	9.54 %	0.74 Rf	26.9 AU	15126.5 AU	14.73 %
12	0.74 Rf	126.9 AU	0.79 Rf	235.8 AU	8.60 %	0.83 Rf	42.1 AU	11625.7 AU	11.32 %
13	0.83 Rf	142.5 AU	0.85 Rf	167.9 AU	6.13 %	0.93 Rf	99.6 AU	9958.5 AU	9.70 %
14	0.93 Rf	100.0 AU	0.97 Rf	242.1 AU	8.84 %	1.00 Rf	0.0 AU	6944.2 AU	6.76 %

Table – 3: Rf value of CNSO at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	0.3 AU	0.07 Rf	12.2 AU	2.08 %	0.09 Rf	0.4 AU	130.9 AU	0.63 %
2	0.13 Rf	3.5 AU	0.16 Rf	11.0 AU	1.87 %	0.17 Rf	9.1 AU	193.3 AU	0.93 %
3	0.17 Rf	9.4 AU	0.18 Rf	12.2 AU	2.07 %	0.20 Rf	1.0 AU	240.7 AU	1.16 %
4	0.24 Rf	6.1 AU	0.32 Rf	71.8 AU	12.24 %	0.33 Rf	62.2 AU	2068.4 AU	9.97 %
5	0.36 Rf	83.3 AU	0.39 Rf	217.5 AU	37.10 %	0.45 Rf	20.2 AU	7412.2 AU	35.73 %
6	0.51 Rf	19.8 AU	0.56 Rf	58.3 AU	9.94 %	0.63 Rf	10.0 AU	3065.8 AU	14.78 %
7	0.64 Rf	8.1 AU	0.70 Rf	29.9 AU	5.10 %	0.73 Rf	23.4 AU	1457.7 AU	7.03 %
8	0.76 Rf	18.1 AU	0.81 Rf	49.3 AU	8.40 %	0.82 Rf	46.6 AU	1551.5 AU	7.48 %
9	0.86 Rf	51.2 AU	0.88 Rf	71.1 AU	12.13 %	0.94 Rf	35.9 AU	3404.0 AU	16.41 %
10	0.94 Rf	36.7 AU	0.96 Rf	53.0 AU	9.05 %	0.99 Rf	0.6 AU	1221.8 AU	5.89 %

Table – 4: GC-MS profile of CNSO

Pk	Peak name	Molecular formula	Molecular weight	Retention time	Area	Area%
01	2-phenylbutyramide, N-propyl-	$C_{13}H_{19}NO$	205.296	6.151	33929	1.17
02	1-methyl-5-oxo-2(3-pyridinyl)	$C_{11}H_{12}N_2O_3$	220.225	11.055	10509	0.36
03	Molybdenum, Tris	MoS_3	192.13	11.100	15187	0.53
04	Octanoic acid, methylester	$C_9H_{18}O_2$	158.238	11.194	22036	0.76
05	4,4,Dimethyl-cyclohex-2-	$C_8H_{12}O$	124.183	11.299	12238	0.42
06	2H-[1,2,4]triazolo[3,4	$C_{12}H_{11}N_5O_2$	257.248	12.259	15842	0.55
07	1H-Isoindol-1-1, Phenylenediimino	$C_{22}H_{12}C_{12}N_4O_2$	453.26	13.477	16027	0.55
08	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	183.295	17.276	39427	1.36
09	Tricyclo[4.3.1.1 undec-3	$C_{13}H_{24}$	180.37	22.875	18359	0.64
10	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.344	23.039	113005	3.91
11	Cyclopenta [C]furo	$C_{17}H_{14}O_6$	314.289	23.646	25242	0.87
12	Phenol	C_6H_5OH	94.113	28.077	14751	0.51
13	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242.403	28.333	77896	2.70
14	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.450	33.184	593091	20.53
15	9-Hexadecenioc acid	$C_{17}H_{32}O_2$	268.441	33.697	202835	7.02
16	9,12-hexadecadienoicacid, methyl ester	$C_{17}H_{30}O_2$	266.425	34.993	84551	2.93
17	9,12,15octadecatrienoicacid, methylester	$C_{19}H_{32}O_2$	292.456	36.212	107654	3.73
18	Methyl stearate	$C_{19}H_{38}O_2$	298.511	37.653	194589	6.74
19	9-octadecenoic acid(Z) methyl ester	$C_{19}H_{36}O_2$	296.487	38.002	631864	21.87
20	9-Octadecenoic acid	$C_{19}H_{34}O_2$	294.479	38.154	233516	8.08
21	m-Fluoro-2-diazoacetophenone	$C_9H_{10}FNO_2$	183.18	38.623	11847	0.41
22	9,12-octadecadienoic acid(Z,Z)- methyl ester	$C_{19}H_{34}O_2$	294.472	38.939	216438	7.49
23	9,12,15- octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.456	40.255	57020	1.97
24	6,6-bis-ethylsulfanyl-4-methyl-hexane-1,2,3,5-tetraol	$C_9H_{10}OS_2$	198.298	43.500	27977	0.97
25	2,7 nonadienedioic acid, 5,5-dicyano- diethyl ester	C_9H_{16}	124.223	44.459	14732	0.51
26	Phenol,3-octyl	$C_{14}H_{22}O$	206.324	45.378	28548	0.99
27	Diisopropoxy-bis-titanium	$C_{16}H_{22}O_6TI$	364.26	45.670	16473	0.57
28	4,4-dimethyl-2-chromanol	$C_{21}H_{46}O_2$	402.653	45.955	14201	0.49
29	Spiro[3,5-ethano-6H-1-pyrindine	$C_{16}H_{10}CIN2O_2$	286.263	49.815	26868	0.93
30	N-(3'3'-dimethylindolin-2'-on-1'-yl)-5-chlorodiclofenac	$C_{10}H_{13}N$	147.216	57.212	12203 2888855	0.42 100.00

the effect of Unsaturation on its biological activities. Pharmaceuticals.16 Mar 2017.

SetiantoWB, Smith RC, Inomata H and Arai K,(2001) Processingof Cashewnut (*Anacardium occidentale*) and Cashew nut shell liquid with supercritical carbondioxide and water; The Journal of Supercritical Fluids,Vol 15:229.

Trevisan MT, Pfundstein B, Harbner R, Wurtele G, Spiegelhalda B, Bartschtt, Owen RW, (2006). Characterization of alkyl phenols in Cashew (*Anacardium occidentale)* products assay of their antioxidant capacity. Food Chem. Toxicol. 44,188-197.

WuY., He L Zhangl, Chen J, Yiz, Zhang J lium Pang X, (2011). Anacardic acid(6- Pentadecylasalicylic Acid) inhibits Tumour Angiogenesis by targeting Src/Fak/Rho GT Pases Signalling pathway. J. Pharmacol. Exp.Ther.339:403-411.