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RESEARCH PAPER

Ultrasonic Assisted Extraction and Gas Chromatography-Tandem Mass Spectroscopy (GC-MS/MS) Analysis of Extracts from Four *Cassia* Species

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Abstract

Ultrasonic assisted extraction (UAE) process increases the rate of extraction, the yield of extracted components and reduces extraction time. The high sensitivity of GC-MS/MS provides reliable identification of phytoconstituents present in a plant extract. This research is to develop an easy and effective method for the extraction and analysis of phytochemicals in *C. singueana*, *C. sieberiana*, *C. tora* and *C. occidentalis* using UAE and GC-MS/MS techniques. The crude extracts obtained from the four *Cassia* species under UAE conditions of 50 mins extraction time, 50 ^{o}C extraction temperature and 20 mL/g solvent to sample ratio were analyzed using GC-MS/MS. Thirty-four compounds were identified by comparison of their mass spectra with those in the National Institute of Standards and Technology library, requiring at least 80% similarity. These compounds comprise fatty acids, fatty alcohol, carboxylic acid, long-chain alkanes, diterpenes, triterpenes, sterols and anthraquinones. *n*-Hexadecanoic acid and phytol were identified in all the *Cassia* species while anthraquinone was detected only from the methanol extracts of *C. tora* and *C. occidentalis.* The bioactivities reported in the literature for these compounds corroborate with the phytoconstituents identified in these plants and support the ethnomedicinal uses of these *Cassia* species.

Keywords: *Cassia*; GC-MS/MS; phytoconstituents; extraction; fatty acid

INTRODUCTION

Cassia (Fabaceae) is well known for its pharmacological and phytochemical diversity. *Cassia* species are popular in folk medicinal uses and has shown anti-inflammatory, antioxidant, anti-plasmodial, hypoglycemic, larvicidal, hyperglycemic, antimutagenic and anticancer activities. The extensive ethnomedicinal applications of *Cassia* species could be attributed to the synergistic activity of the phytoconstituents (Harshai and Sanjivani, 2013). *C. singueana*, *C. sieberiana, C. tora* and *C. occidentalis* are native medicinal plants with diverse medicinal applications common among traditional medicine practitioners in Africa. They are used by

traditional herbalists in the treatment of fever, malaria, ulcer, constipation, diarrhea, skin disorder and diabetes mellitus (Schmelzer and Guri-Fakim, 2008; Favour and Madubuike, 2015; Ibrahim and Islam, 2014). *C. tora* is utilized as germicide and pesticide (Smita and Patil, 2010). Previous studies have shown that both *C. singueana* and *C. sieberiana* possess antioxidant, antimalarial, anti-diarrheal and anti-ulcer activities (Onakpa and Ode, 2010; Yadav et al., 2010; Jibril et al., 2017; Ifeanyi and Ode, 2012). Phytochemicals which include anthraquinones, triterpenes, sterols, and flavonoids have been reported from these *Cassia* species (Khomendra et al., 2014; Jibril et al., 2017; Ode and Asuzu, 2014). The various biological activities such as antioxidant, anti-inflammatory, antimicrobial, anticholinesterase and anticancer properties displayed by plant extracts could be attributed to the phytochemicals present in these plants (Cai et al., 2006). However, the method of extraction and the techniques used in profiling the phytochemicals in the extracts is important in the search for the potential novel drug from plant extracts. Hence, it is important to screen plant extract using modern techniques such as ultrasonic-assisted extraction (UAE) and the use of a hyphenated system like GC-MS/MS to reduce the duration used in the search for new drugs from medicinal plants.

The conventional methods used in the extraction of *C. singueana*, *C. sieberiana*, *C. tora* and *C. occidentalis* are maceration and soxhlet extraction techniques (Ibrahim and Islam, 2014; Gideon et al., 2015; Gati et al., 2015; Vedpriya and Yadav, 2010). Although one report on the optimization of UAE of *C. singueana* showed the efficiency of UAE in the extraction of *C. singueana* (Jibril et al., 2018). The conventional extraction methods used for the extraction of *Cassia spp.* are time-consuming and also requires a relatively large amount of organic solvents (Lijun and Curtis, 2006). Alternative and modern extraction techniques such as ultrasonic-assisted extraction (UAE) has been developed to shorten the extraction time, reduce solvent consumption, increase extraction yield, and prevent environmental pollution by organic solvents (Toma et al., 2001). The UAE technique provides cavitation which allow solvent penetration into the plant sample, increasing the solvent-sample contact and improving mass transfer of phytochemicals from the plant sample into the solvent (Guardia and Armenta, 2011). Beside development of efficient extraction technique, the isolation of the bioactive compound is still a challenge. Therefore, screening of the crude extract for the presence of known compounds prior to isolation process could significantly reduce the time and save resources. Tandem mass spectrometry is a modern mass spectrometric technique which can be used to investigate complex mixtures such as plant extracts (Busch et al., 1988). The use of GC/MS analysis in the profiling of various extracts from *C. singueana* has been reported (Mohammed et al., 2013). However, the Gas Chromatography tandem mass spectroscopy (GC-MS/MS) technique is more sensitive than the use of Thin-layer Chromatography (TLC) or the tedious column chromatography (CC). Moreover, the coupled mass spectrometry in the GC-MS/MS system will enhance the easy identification of the phytochemicals.

In this study, the leaf of *C. singueana*, *C. sieberiana* and *C. tora*; the stem of *C. tora* and the whole plant of *C. occidentalis* were extracted in various solvents (*n*-hexane, EtOAc and MeOH) using UAE technique. The extracts obtained were subjected to GC-MS/MS analysis to identify phytochemicals present in these plant extracts.

MATERIALS AND METHODS

Plant materials

The leaf part of *C. singueana* (BUKHAN0316), *C. sieberiana* (BUKHAN0065) and *C. tora*; the stem part of *C. tora* (BUKHAN0307) and the whole plant of *C. occidentalis* (BUKHAN0073) were collected in January 2016, from Alkaleri, Bauchi State, Nigeria. The plants were identified by Mr. Baha'uddeen Said Adam of the Department of Plant Biology, Bayero University Kano. The voucher specimens have been deposited at the Herbarium of Department of Plant Biology, Bayero University Kano, Nigeria. All solvents used for GC-MS/MS analyses were analytical grade. The samples were analyzed on Agilent GC-MS/MS equipped with a fused silica capillary column, using an ionization source of 70 eV and fragmentation by electron ionization (EI). Ultrasonic cleaning bath (Elmasonic, Elma Schmidbauer GmbH Germany) with a frequency of 60 kHz and a power of 750 W, equipped with time and temperature controller.

Ultrasonic-assisted extraction of *Cassia* species

Air-dried powdered sample of *C. singueana* (5 g), *C. sieberiana* (5 g), *C. tora* (5 g) and *C. occidentalis* (5 g) were extracted separately in *n*-hexane (100 mL), EtOAc (100 mL) and MeOH (100 mL) using UAE under the conditions of 50 min extraction time, 50 °C extraction temperature and 20 mL/g solvent to sample ratio. The extract was filtered and the solvent was removed using a rotary evaporator to obtain crude *n*-hexane extract, EtOAc extract and MeOH extract for the various plant samples. The samples were allowed to dry completely and the weight of each sample was taken and recorded.

Gas chromatography coupled to mass spectrometry analyses (GC-MS/MS) of *Cassia* spp. extracts and identification of compounds

The crude extracted powder sample was suspended in MeOH to obtain a concentration of 100 mg/mL (w/v) followed by filtration using a microfiltration membrane (0.45 µm). Aliquot (1 µL) of the prepared sample was employed for GC-MS/MS analysis. The GC-MS/MS analysis was carried out using Agilent Technologies GC-MSMS (GCQQQ) with fused silica (15m×0.2mm ID×1µm) of the capillary column. The instrument was set to an initial temperature of 110 ºC and maintained at this temperature for 2 min. At the end of this period, the oven temperature was rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1 mL/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. The mass spectral scan range was set at 30-460 (m/z). Using computer searches on a National Institute Standard and Technology NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS/MS compounds present in the plants' sample were identified, requiring at least 80% similarity. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

RESULTS AND DISCUSSION

Among the parameters that affects the UAE techniques include the extraction time, extraction temperature and solvent to sample ratio. The extraction temperature greatly influence the solvent to sample contact and the mass transfer (Dominguez and González, 2018). The various extracts obtained by UAE with *n*-hexane, EtOAc and MeOH from *C. singueana*, *C. sieberiana*, *C. tora* and *C. occidentalis* were analyzed by GC-MS/MS and mass spectra of the

compounds were compared with the mass spectra in NIST mass spectra (MS) library. The use of UAE technique will increase the extraction yield and also enhance the quality of extracts obtained compared to the conventional methods of extraction (Lijun and Curtis, 2006). Table 1 shows the sample label and extraction yield obtained from *C. singueana*, *C. sieberiana*, *C. tora* and *C. occidentalis* under UAE conditions of 50 min extraction time, 50 ^{o}C extraction temperature and 20 mL/g solvent to sample ratio.

Table 1. Ultrasonic-Assisted Extraction (UAE) of *Cassia* species in *n*-hexane, EtOAc and MeOH.

Plant	Part	Extraction Solvent	Sample label	Yield (g)
Cassia singueana	Leaf	*n*-hexane	ALH	0.40
		EtOAc	ALE	0.60
		MeOH	ALM	0.90
Cassia sieberiana	Leaf	*n*-hexane	BLH	0.67
		EtOAc	BLE	0.70
		MeOH	BLM	0.85
Cassia tora	Leaf	*n*-hexane	CLH	0.40
		EtOAc	CLE	0.75
		MeOH	CLM	0.55
	Stem	*n*-hexane	CSH	0.45
		EtOAc	CSE	0.43
		MeOH	CSM	0.85
Cassia occidentalis	Whole plant	*n*-hexane	DPH	0.38
		EtOAc	DPE	0.90
		MeOH	DPM	0.80

All the extracts obtained from the four *Cassia* species by UAE system were analyzed by means of GC-MS/MS technique. From the extracts obtained using three different solvents, thirty-four compounds were identified which include fatty acids, fatty alcohol, carboxylic acid, long-chain alkanes, diterpenes, triterpenes, sterols and anthraquinones. The identified compounds in each extract of these *Cassia* species are presented in Table 2.

Each extract obtained from the different solvents displayed diversity in metabolite. The MeOH extracts obtained contained more polar constituents whereas the less polar extracts obtained with *n*-hexane contained high levels of long-chain hydrocarbons, fatty acids, fatty alcohols, triterpenes, sterols, and other lipophilic compounds. The EtOAc extracts obtained contained less complex but moderate to less polar compounds. Some of the compounds were identified in all the four *Cassia* species. *n*-Hexadecanoic acid was identified in the EtOAc leaf extracts of *C. singueana, C. sieberiana, C. tora,* and the EtOAc whole plant extract of *C. occidentalis*. It is also identified in the *n*-hexane and EtOAc stem extract of *C. tora.* Phytol was identified in the leaf extract of *C. singueana, C. sieberiana, C. tora* obtained with *n*-hexane and EtOAc; and the *n*-hexane whole plant extract of *C. occidentalis.* The anthraquinone compounds were identified only in the MeOH extracts of *C. tora* and *C. occidentalis.* The compounds identified from extracts of these *Cassia* species have been reported to show various biological activities.

n-Hexadecanoic acid has antioxidant, nematicide and pesticidal properties. The antinociceptive and antioxidant activities of phytol have been reported (Camila et al., 2013). Squalene has demonstrated antioxidant and antitumor activities (Zih-Rou et al., 2009). The study has shown that stigmasterol facilitates cholesterol elimination in mice (Lifset, 2018). Furthermore, stigmasterol has demonstrated antioxidant, antitumor, anti-hypercholesterolemic, cytotoxic, and CNS effects (Navpret et al., 2011).

Table 2. Ultrasonic-Assisted Extraction (UAE) of *Cassia* species in *n*-hexane, EtOAc and MeOH.

R_t (min)	MS (%)	MF	Compound name	*Cassia spp* extract
12.513	89.0	C_8H_8O	Benzofuran, 2,3-dihydro-	ALM
13.449	98.7	$C_6H_6O_2$	Resorcino	ALM
15.053	83.6	$C_{13}H_{26}$	6-Tridecene, (*Z*)-	ALM
16.636	96.6	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol	BLE, BLH, CLH, CLE
16.952	80.3	$C_{14}H_{22}O_2$	3-buten-2-ol, 2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxotricyclo[5.2.1.0(2,4)]dec-9-yl) ethanone	DPM
18.565	82.6	$C_7H_{14}O_6$	Myo-Inositol, 4-C-methyl-	ALM, BLM
18.877	85.4	$C_{17}H_{36}$	Tetradecane, 2,6,10-trimethyl	DPH
18.549	84.4	$C_{15}H_{24}O$	Cis-Z-, alpha, bisabolene expoide	DPM
19.797	82.3	$C_{17}H_{32}O_2$	7-methyl-Z-tetradecen-1-ol acetate	DPM, CLM,
20.328	95.1	$C_{20}H_{38}$	Neophytadiene	ALE, BLE, CLE
20.311	88.3	$C_{22}H_{42}O_2$	Phytol, acetate	CLM, CSE, DPE,
21.550	97.7	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	ALE, BLE, DPE, CLM, CLE, CSH, CSE
21.181	84.3	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	ALH
21.434	91.0	$C_{18}H_{28}O_3$	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	BLM
21.533	87.7	$C_{18}H_{24}O$	Estra-1,3,5-(10)-teien-17, beta,-ol	DPH
21.924	96.9	$C_{11}H_{14}O_4$	Trans-Sinapyl alcohol	CSM
23.000	93.9	$C_{20}H_{40}O$	Phytol	ALH, BLH, BLE, DPH, CLE
23.585	80.0	$C_{14}H_{15}NO$	benzenamine, 4-[2-(2-furanyl) ethenyl]-N,N-dimethyl-	BLM
23.172	94.8	$C_{18}H_{32}O_2$	9,12-Octadecanoic acid	DPE, CSM
23.203	90.7	$C_{18}H_{34}O_2$	*cis*-Vaccenic acid	DPM, CLH
23.289	93.4	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	CLM, ALE
23.378	89.4	$C_{18}H_{34}O_2$	Oleic acid	CSE, CSH,
25.152	95.9	$C_{15}H_{10}O_4$	9,10-anthracenedione, 1,8-dihydroxy-3-methyl	DPM, CSM
28.726	92.4	$C_{30}H_{50}$	Squalene	BLE, BLH, DPE, CLE
30.542	83.2	$C_{31}H_{50}O_2$	Stigmasta-5, 22-dien-3-ol, acetate, (3.beta.)-	CLH, CSH
31.256	83.3	$C_{29}H_{50}O_2$	dl-α-Tocopherol	BLE, BLH
31.066	80.0	$C_{29}H_{48}$	Stigmastan-3,5-diene	CSH, CLH
32.774	91.6	$C_{29}H_{48}O$	Stigmasterol	BLE, BLH, DPH, DPE, CLE, CLH, CSE
33.510	86.2	$C_{29}H_{50}O$	γ-Sitosterol	BLE
33.483	83.3	$C_{29}H_{50}O$	β-Sitosterol	DPE
34.562	83.9	$C_{29}H_{46}O$	4,22-Stigmastadiene-3-one	CSE
34.406	87.7	$C_{30}H_{48}O$	Lup-20(29)-en-3-one	BLH, BLE, DPH
35.491	80.0	$C_{29}H_{48}O$	β-Sitostenone	DPH
35.495	82.1	$C_{29}H_{48}O$	γ-Sitostenone	CSE

A = *C. singueana*; B = *C. sieberiana*; C = *C. occidentalis*; D = *C. tora*; L = leaf; S = stem; P = whole plant; H = *n*-hexane; E = ethyl acetate; M = methanol; Rt = Retention time; MS = similarity base on NIST MS database; MF-molecular formula

CONCLUSION

The UAE technique has demonstrated the use of less amount of sample and solvent to achieve extraction within a short period of time on *C. singueana*, *C. sieberiana*, *C. tora* and *C. occidentalis*. The GCMS/MS instrument was able to screen thirty-four compounds present in the various extracts obtained from the four *Cassia* species. The UAE process will be more economical for the screening of medicinal plants due to the fact that it reduces the duration and solvent consumption required for the screening process. The GC-MS/MS technique provided more reliable results of the phytochemicals present in these four *Cassia* species. Hence, the extensive ethnomedicinal applications of *C. singueana*, *C. sieberiana*, *C. tora* and *C. occidentalis* could be attributed to the synergistic activity of the phytoconstituents identified in the various extracts from these four *Cassia* species. Similarly, prior to isolation of pure compounds, the UAE and GCMS/MS techniques can be used to avoid reisolation of known compounds.

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