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Molecular, phytochemical and biological investigation of the secondary metabolites of *Sorghum virgatum*

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Abstract
Phytochemical study of the ethanolic extract of *Sorghum virgatum* (Hack.) herb led to the isolation and identification of 10 compounds including; ferulic acid (1), caffeic acid (2), tricin (3), luteolin-3´,4´-dimethyl ether (4), stigmasterol-O-β-D-glucopyranoside (5), daucosterol (6), salcolin A (7), tricin-7-O-β-D-glucopyranoside (8), luteolin-7-O-β-D-glucopyranoside (9), and laempferol-3-O-rutinoside (10). These compounds were isolated for the first time from this species. Structure elucidation of the isolated metabolites was achieved by spectroscopic data (1D and 2D NMR). Antioxidant and cytotoxic activities of isolated compounds were studied and significant results were obtained. The physicochemical properties of the compounds were investigated to predict their human oral absorption, systemic distribution, metabolism, and excretion profiles (ADME). Predictions were performed for CNS activity, total solvent accessible surface area, aqueous solubility, partition coefficients, violations of role of five and role of three, and binding to human serum albumin.

Keywords: *Sorghum virgatum*; molecular study; antioxidants; cytotoxicity and antimicrobial activities

Introduction
Poaceae (Gramineae) are one of the largest vascular plant families, containing from 650 to 785 genera and about 10,000 species. Poaceae are also one of the most ecologically and economically important plant families (Stanely 1999) [1]. Some plants of poaceae are used in folk medicine for their effects as antihypertensive, antidiabetic, anti-inflammatory, anthelmintic, astringent, antiulcer, diuretic and antioxidant agents (Moreira *et al*. 2010; Rathod *et al*. 2011) [2, 3]. Poaceae contain a very wide range of constituents such as foodstuffs, starches, sugar and secondary metabolites including volatile oils, alkaloid, saponins, cyanogenetic substances, phenolic acids, flavonoids, lignans, phenylethanolds, and terpenoids [4]. Sorghum belongs to Poaceae (Graminae) family that is recognized as an important crop throughout the arid tropical and sub-tropical regions of Africa, Asia and Central America. In Africa, most of the grain is used to prepare foods and beverages for human consumption including traditional stiff or thin porridges, granulated foods and beer [4-6]. Sorghum is rich in the phytochemicals known to significantly affect human health, such as tannins, phenolic acids, anthocyanins, phytosterols and policosanols [5]. Recent studies have shown that sorghum has antioxidant activity antimutagenic [7], anticarcinogenic [8] and cholesterol lowering effects [9], and can reduce the risk of cardiovascular disease. *Sorghum virgatum* (Hack.) is perennial, weeds in fields and a long channels, which represented in flora of Egypt by four species [10]. In this study, we are reporting for the first time about the phytochemical and biological investigations of *S. virgatum.*

Material and Methods
General experimental procedures
UV spectra were determined with a Hitachi 340 spectrophotometer, Tokyo, Japan. IR spectra were carried out on a Nicolet 205 FTIR spectrometer connected to a Hewlett-Packard Color Pro Plotte, USA. NMR spectra were recorded on a Varian Mercury (USA) 400 MHz spectrometer at 400 (^{1}H) and 100 MHz (^{13}C) in DMSO-d_6 or $CDCl_3$-*d* solution and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135º). The EIMS spectra were measured using El/MS 502 mass spectrometer having a direct inlet system and operating at 70eV. The ESI/MS spectra were measured using a Bruker Bioapex-FTMS with electrospray ionization (ESI), USA. Column chromatographic separation was performed on silica gel 60

(Si gel 60, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). TLC was performed on pre-coated TLC plates with silica gel 60 F_{254} (0.2 mm, Merck, Darmstadt, Germany). Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C.

Plant material

S. virgatum herb was collected from the surroundings of faculty of pharmacy Al-Azhar University, Cairo, Egypt, in Jun 2012. The plant was authenticated by Dr. Abdu Mareey, Prof. of plant taxonomy, Faculty of Science, Al-Azhar University, Cairo, Egypt. A voucher specimen (SV1) has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation

Air-dried powdered aerial parts of S. virgatum herb (2kg) were exhaustively extracted by maceration with 70 % EtOH (7L x 3) at room temperature. The combined ethanolic extracts were concentrated under vacuum at 40oC to dryness. The concentrated ethanolic extract (450 g) was suspended in distilled water (600 ml) and partitioned successively with n-hexane, ethyl acetate and n-butanol to give 14gm, 10g and 18g, respectively. The ethyl acetate extract was subjected to a silica gel column eluted with n-hexane: ethyl acetate 95:5 to 10:90 to obtain six fractions of A (500mg), B (700mg), C (450mg), D (850mg), E (900g) and F (800mg). Fraction B was re chromatographed on Silica gel CC eluted with n-hexane:ethyl acetate 90:10 to 80:20 to give compound 9 (45mg). Fraction D was chromatographed on a Sephadex LH-20 column eluted with CH2Cl2: MeOH 50:50 to give compounds 7 (20mg) and sub fraction D-1 (90mg). The sub fraction D-1 was further subjected to solid phase extraction (RP-C18) using 50:50-60:40 MeOH: water system to obtain compound 8 (25mg). Fractions E and F were separately subjected to Sephadex LH-20 columns eluted with MeOH to afford compounds 3 (50mg) and 2 (12mg) from fraction E and compounds 1 (8mg) and 10 (30mg) from fraction E. The n-butanol fraction was subjected to vacuum liquid chromatography (VLC) on silica gel eluted with CH2Cl2: MeOH 95:5 to 30:70 to give five fractions of A (1.5g), B (2g), C (1.7g), D (3g) and E (2.2g). Fraction A was subjected to column chromatography on Silica gel eluted with CH2Cl2: MeOH 95:5 to give three sub fractions of A-1 (300mg), A-2 (250mg) and A-3 (430mg). Sub fraction A-2 was purified on a Sephadex LH-20 eluted with MeOH to obtain compound 4 (20mg). Fraction B was repeatedly chromatographed on silica gel columns eluted with CH2Cl2: MeOH 95:5 to 80:20 and Sephadex LH-20 eluted with MeOH to afford compounds 6 (40mg) and 5 (30mg), respectively.

Conformational search

The 3D molecular structures were constructed in Maestro [11] using OPLS2005 force field and charges. The conformational space of each molecule was searched using the mixed torsional/low-mode sampling method of Macromodel [12] with intermediate torsion sampling. For each rotatable bond, 100 steps were used. An energy window of 10 kcal/mol was employed as a maximum window between the lowest and highest energy conformers. We ensured the elimination of all redundant conformers by setting a root mean square deviation (RMSD) of less than 0.5 Å. The Powell-Reeves conjugate gradient (PRCG) method was used for the final geometry optimization step.

Molecular properties calculation

A prediction for human oral absorption, systemic distribution, metabolism, and excretion (ADME) of the compounds was performed by QikProp [13]. QikProp was used to calculate the compounds' CNS activity, total solvent accessible surface area, aqueous solubility, partition coefficients, violations of role of five and role of three, and binding to human serum albumin.

Cytotoxicity assays

The cytotoxicity of the isolated compounds were tested against three human tumor cell lines; Hepatocellular carcinoma cells (HepG-2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7). The cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) supplemented with 10% inactivated fetal calf serum and 50µg/mL gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO2 and were sub cultured two to three times a week. The cytotoxic activity was determined by using cell viability assay method as described previously [14, 15]. The experiments were performed in triplicates and the percentage of cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells. Concentration-response curves were prepared and the IC50 values were determined.

Results and Discussion

The ethanolic extract of *S. virgatum* herb aerial parts resulted in the isolation and identification of ten compounds (Fig. 1) for the first time from the plant and identified by comparison of their spectroscopic data with the corresponding literature which showed complete resemblance with those reported. The isolated metabolites were identified as Ferulic acid (1), Caffeic acid (2), Tricin (3), Luteolin-3`,4`-dimethyl ether (4), Stigmasterol-*O*-*β*-D-glucopyranoside (5), Daucosterol (6), Salcolin A (7), Tricin-7-*O*-*β*-*D*-glucopyranoside (8), Luteolin-7-*O*-*β*-*D*-glucopyranoside (9), Kaempferol-3-*O*-rutinoside (10). [16-27]

Compounds 1–10 were evaluated for their cytotoxic activity, compound 7 exhibited good cytotoxic activity against the tested cell lines, the rest of the isolated compounds showed moderate cytotoxic activity (Table 2).

Compounds 1, 2, 3 and 4 are structurally rigid having 12, 18, 12, and 12 conformers, respectively. This is attributed to the small number of rotatable bonds (Fig. 2). Other compounds (5-10) are highly flexible expressing high number of rotatable bonds (Figure 2). Compounds (3-10) showed intra-molecular hydrogen bonds either in the main skeleton or in the sugar moiety (Fig. 3).

Compounds 1 and 2 did not show this kind of hydrogen bonds due to lacking of the proper bond distance and angle between the hydrogen bond donor and acceptor groups. Compound 10 showed hydrogen bonds between the sugar and the main skeleton, which made the structure to have a folded shape (Fig. 3). Compounds 5, 6, 8 and 9 did not have hydrogen bonds between the sugar and the main skeleton. The calculations identified a number of reactive functional groups that can lead to toxicity problems.

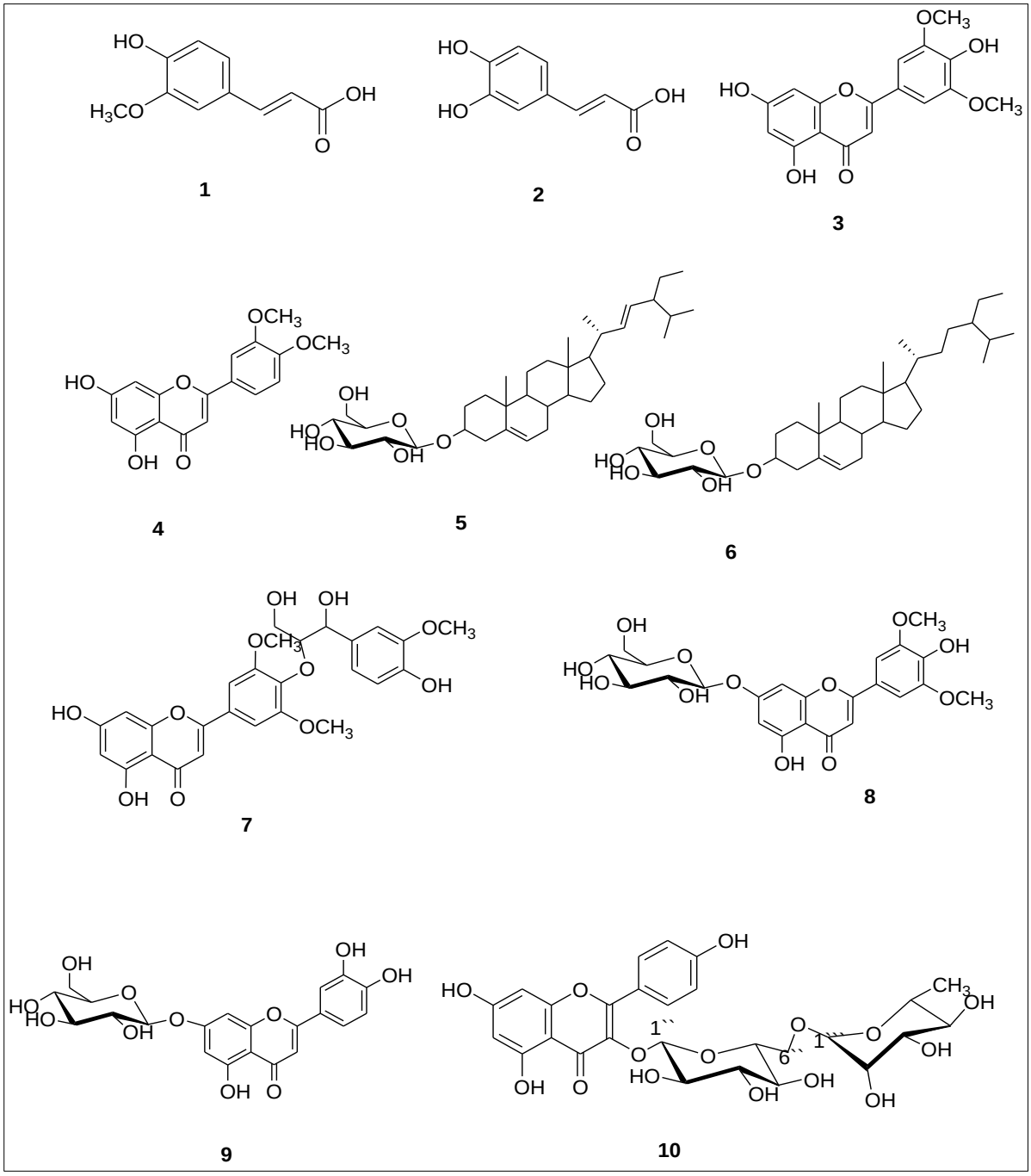

Fig 1: Compounds 1-10

Aromatic hydroxylation and alcohol oxidation of the chemical structures are highly expected. Because of the identified reactivity, we considered comparing these compounds with already USFDA approved drugs (Table 1). All compounds are presumed to be CNS inactive. Compounds 5 and 6 are expected to significantly bind to human plasma albumin. The predicted percent human oral absorption of these compounds is more than 50% except for compounds 8, 9 and 10.

Table 1: Percent structural similarity of compounds 1, 2, and 3 with USFDA approved drugs.

Compound 1		**Compound 2**		**Compound 3**	
Approved Drug	%S*	Approved Drug	%S*	Approved Drug	%S*
Idrocilamide	92	Styramate	92	Aminaftone	90
Ibuproxam	91	Hydroxyphenamate	90	Lansoprazole	87
Hydroxyphenamate	91	Dobesilate	89	Warfarin	87
Aminometradine	90	Ethamsylate	89	Dantrolene	87
Actarit	90	Aminosalicylic	88	Sulfaphenazole	86
Procodazole	89	Mesalamine	88	Voriconazole	86
Iproclozide	89	Etilefrine	86	Omeprazole	86
Dimecrotic	89	Baclofen	86	Ditazole	86
Phenprobamate	88	Secnidazole	86	Fluconazole	85
Clorprenaline	88	Proxibarbal	85	Rosiglitazone	85

* %Similarity

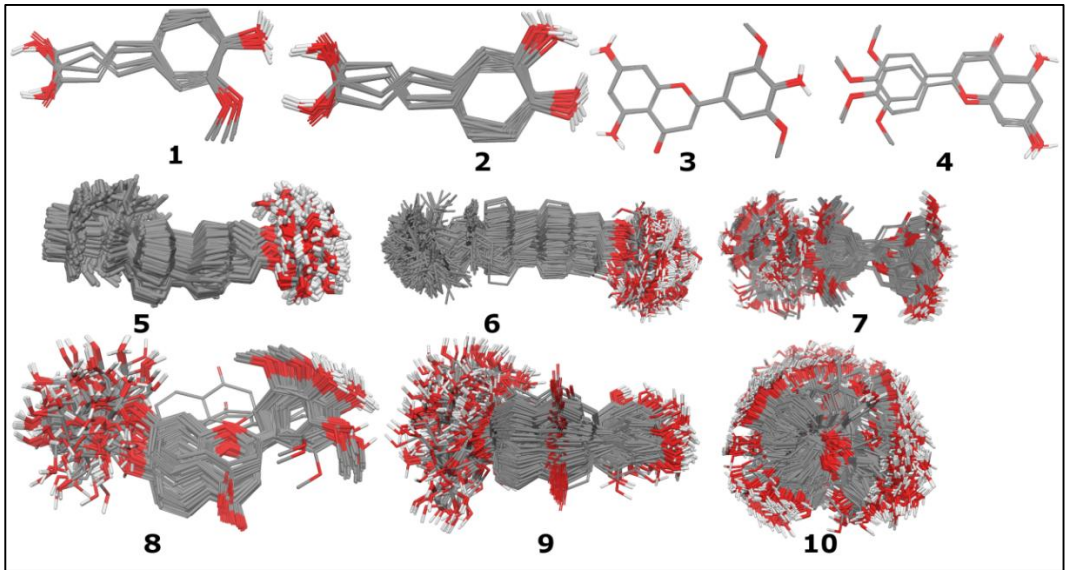

Fig 2: The generated conformers at 10 kcal/mol energy window. An alignment is made for each compound to show the major structural states.

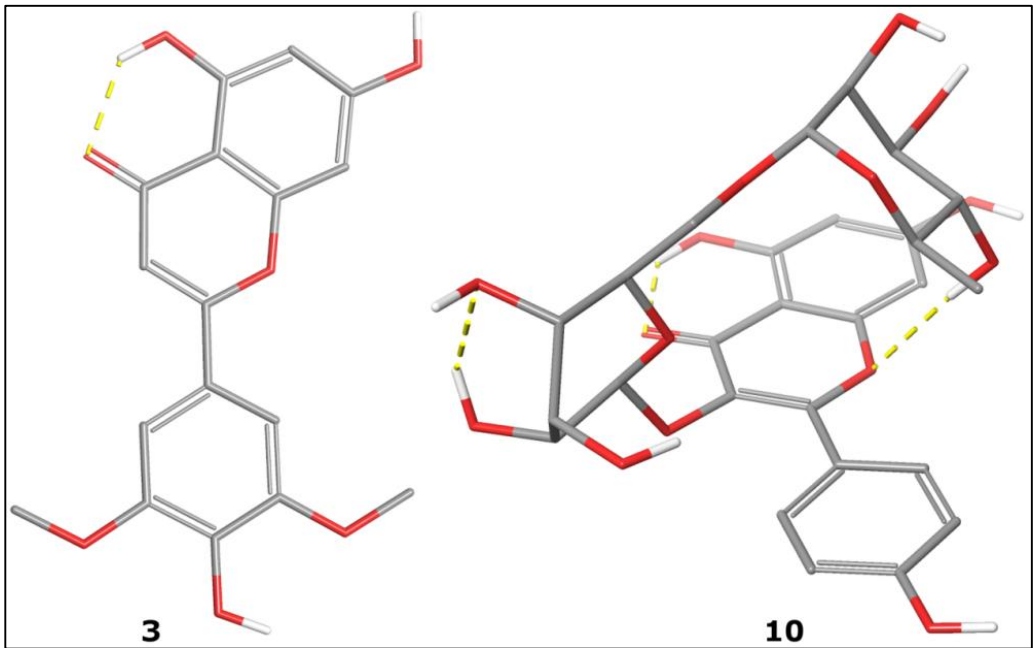

Fig 3: The intermolecular hydrogen bonding that stabilizes the structures.

Table 2: Cytotoxicity of isolated compounds against Hepatocellular carcinoma cells (HepG-2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7).

	IC_{50} (µg/ml)		
Extract/compound	**Breast carcinoma (MCF-7)**	**Colon carcinoma (HCT-116)**	**Hepatocellular carcinoma (HepG-2)**
1	71.3	35.2	32.5
2	56.6	22.5	29.1
3	**42.3**	**15.1**	**22.7**
4	95.2	64.2	75.6
7	31.3	29.5	35.2
8	45.1	18.4	32.6
9	>100	68.4	80.1
10	92.3	41.9	53.6

Values are presented as mean ± SE of 2 test sample observation, compared with that of control group ($p < 0.05$) for all values. IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number

Conclusion

10 compounds were isolated and identified from *Sorghum virgatum*, antioxidant and cytotoxic activities of isolated compounds were studied and significant results were obtained. The physicochemical properties of the compounds were investigated to predict their human oral absorption, systemic distribution, metabolism, and excretion profiles (ADME). Predictions were performed for CNS activity, total

solvent accessible surface area, aqueous solubility, partition coefficients, violations of role of five and role of three, and binding to human serum albumin.

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