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Research Article

CYTOTOXIC, ANTIMICROBIAL AND ANTIVIRAL EVALUATION OF THE AERIAL PARTS OF *SORGHUMVIRGATUM*HACK

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ABSTRACT

Sorghum virgatum (Hack) belongs to Poaceae (Graminae) family, which represented in flora of Egypt by four species. It worth noting that nothing was reported about phytochemical and biological investigation of *S.virgatum*. Therefore, phytochemical and biological investigation of the different extracts of *S. virgatum*isimportant. Sorghum is rich in phytochemicals known to significantly affect human health. Cytotoxic, antiviral and antimicrobial activities of different plant extracts were studied and significant results were obtained.

Key words:*Sorghumvirgatum*(Hack); antiviral, cytotoxicity and antimicrobial activities.

Introduction:

A big chunk of the world populationespecially, in developing countries depends on the traditional system ofmedicine. Herbal preparationsare sources of very potent and powerful drugs.The World HealthOrganization reported that up to80% of the world's population relies on traditional medicine and a major part ofthe traditional therapies involves the use of plant extractsor their active constituents (1).

Although antibiotics have saved billions of lives and played an important role in human history. Unexpectedly, many pathogenic microorganisms, e.g. methicillin resistant *Staphylococcus aurous* (MRSA) and vancomycin-resistant *Enterococcus faecium*, have developed resistance towards current antibiotics and this trend has become more and more serious. Poaceae(Gramineae) are one of the largest vascular plant families, containing 10,000 species (2). Some plants of poaceae used in folk medicine for hypertension, antidiabetic, anti-inflammatory, anthelmintic, astringent, antiulcer, diuretic and antioxidant (3, 4). *S.virgatum*belongs to Poaceae (Graminae) family is perennial, weeds in fields and long channels, which represented in flora of Egypt by four species (5).

It worth noting that nothing was reported about phytochemical and biological investigation of*S.virgatum.*Therefore, phytochemical and biological investigation of the different extracts of*S.virgatum*lends support to the ethno medicinal use of the plant in combating microbial infections and viral infections.

Experimental

General experimental procedures; UV spectra were determined with PyeUnicam spp. 1750 spectrophotometer. Si gel (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia) were used for open column chromatography. Solid phase extraction was performed on SPE-C_{18} cartridges (A Strata column, Phenomenex, USA). TLC was carried out on pre-coated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min, or spraying with ammonia or aluminum chloride solutions.

Plant material

*S.virgatum*herb was collected from the surroundings of faculty of pharmacy Al-Azhar University, Cairo, Egypt, in Jun 2014. The plant was kindly identified by Professor AbdoMareyProf. of taxonomy, Botany Department, Faculty of Science Al-Azhar University, Cairo, Egypt. A voucher specimen has been deposited in the

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Extraction and isolation:

Air-dried powdered aerial parts of *Sorghumvirgatum*herb (1kg) were subjected to exhaustive extraction with 70% ethanol (7Lx3). The combined ethanolic extracts were concentrated under vacuum at 40^{o}C 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 14 g, 10g and 18g, respectively.

Antiviral assays

The screening antiviral assay system using cytopathic effect inhibition assay at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University. This assay was selected to show specific inhibition of a biologic function, i.e., cytopathic effect (CPE) in susceptible mammalian cells (6). In brief, monolayers of 10,000 vero cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24h at 37ºC in a humidified incubator with 5%CO_2. The plates were washed with fresh DMEM and challenged with 10^4 herpes simplex type 2 virus doses and simultaneously the cultures were treated with two-fold serial dilutions of tested compoundin fresh maintenance medium and incubated at 37ºC for two days. An infection control as well as untreated Vero cells control was made in the absence of tested compound. Six wells were used for each concentration of the tested compound. Every 24 h the observation under the inverted microscope was made until the virus in the control wells showed complete viral-induce cytopathic effects (CPE). Antiviral activity was determined by the inhibition of cytopathic effect compared to control, *i.e*., the protection offered by the tested compound to the cells was scored (7). The monolayers were fixed with formalin then stained with a 0.1% crystal violet solution and digital photos were taken using Olympus inverted microscope Model CKX41. Three independent experiments were assessed each containing four replicates per treatment. Acyclovir, which is clinically used for the treatment of herpetic viral disease, was used as a positive control under this assay system.

Antimicrobial assays

Antimicrobial activities of *n*-hexane, ethyl acetate and *n*-butanol fractions of *Sorghumvirgatum*herb were investigated in *vitro* against different bacteria and fungi using the diffusion agar technique according toBauer et al., 1966(8). The following bacterial strains were employed in the screening: Gram-positive bacteria; *Staphylococcus aureus*(RCMB 010028) and*Bacillissubtilis*(RCMB 010067), Gram-negative bacteria; *Escherichia coli* (RCMB 010052) and *Pseudomonas aeruginosa*(RCMB 010043). As fungal strains *Aspergillus fumigates* (RCMB 02568)and *Candida albicans*(RCMB 05031). Ampicillin, Gentamycin and Amphotericin B were used as reference drugs. The microbial species are environmental and clinically pathogenic microorganisms obtained from Regional Center for Mycology and Biotechnology antimicrobial unit (RCMB), Al-Azhar University.

Cytotoxicity assays

The cytotoxicity of the *n*-hexane, ethyl acetate and *n*-butanol fractions 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% CO_2 and were sub cultured two to three times a week. The cytotoxic activity was determined by using cell viability assay method as described previously (9, 10)**.** 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 IC_{50} values were determined.

Results and discussion:

*S.virgatum*herb aerial parts were extracted with alcohol and the dried alcoholic extract was suspended in water and fractionated with *n*-hexane, ethyl acetate and *n*-butanol. Cytotoxic, antiviral and antimicrobial activities of *n*-hexane, ethyl acetate and *n*-butanol fractions were evaluated.The ethyl acetate and *n*-butanol extracts of *S.virgatum*showed weak antiviral effects against HAV-10 and showed no activity against HSV-1 and

HSV-2. The *n*-hexane extract showed no antiviral activity against all viruses tested (Table 1). The ethyl acetate and *n*-butanol extracts of *S.virgatum*demonstrated variable antimicrobial activity against most of the specific organisms tested (Table 2). The ethyl acetate extract was the most active against *C. albicans* and *E. coli* compared to that of *n*-butanol. The *n*-hexane showed no antimicrobial activity against all microorganisms tested. The ethyl acetate and *n*-hexane of *S.virgatum*were the most active extracts as cytotoxic agents against the tested cell lines with values of IC_{50} from 6.1 to 9.6µg/ml compared to that of *n*-butanol (Table 2).

This study provides an evidence for the strong cytotoxic activity of the ethyl acetate and *n*-hexane extracts of *S.virgatum*(Table3). In addition to the highest antimicrobial activity of the ethyl acetate extract ofthe plant against *C. albicans* and *E. coli* (Table 2) that could be considered a valuable medicinal plant species. The higher activities of the ethyl acetate extract may due to the flavonoid contents which were reported previously. Additionalstudies are needed to identify the constituents of *n*-hexane extract that are responsible for its higher activity.

Table 1: Antiviral activity of *Sorghumvirgatum*using CPE inhibition assay:

Plant extract	HAV-10	HSV-1	HSV-2
n-hexane	-ve	-ve	-ve
Ethyl acetate	+	-ve	-ve
n-butanol	+	-ve	-ve

+: Weak antiviral effect; -ve: No antiviral activity.

Table 2: Antimicrobial activity of *Sorghumvirgatum*using agar diffusion method:

Organisms	Diameter of inhibition zone (mm)			
	n-hexane	Ethyl acetate	*n*-butanol	Standards
Fungi				Amphotericin
Aspergillusfumigatus(RCMB 02564	NA	16.2±0.44	NA	22.9±0.44
Candida albicans (RCMB 05035	NA	20.9±0.25	NA	21.4±0.25
Gram +ve				Ampicillin
Staphylococcus aureus(RCMB 010027**)**	NA	16.9±0.58	15.4±0.25	28.9±0.14
Staphylococcus epidermidis(RCMB 010024)	NA	20.9±0.25	17.9±0.37	28.3±0.37
Gram -ve				Gentamycin
Pseudomonas aeruginosa(RCMB 010043	NA	15.2±0.58	11.1±0.37	20.3±0.37
Escherichia coli (RCMB 010056)	NA	17.1±0.25	12.6±0.63	21.4±0.25

Well diameter: 6.0 mm …. …. (100µl was tested), Sample concentration (20mg/ml),
NA: No activity, data are expressed in the form of mean ± Standard deviation.

Table3: Cytotoxicity of *S. virgatum*extractsagainst Hepatocellular carcinoma cells (HepG-2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7):

IC_{50} (µg/ml)			
Extract	**Breast carcinoma (MCF-7)**	**Colon carcinoma (HCT-116)**	**Hepatocellular carcinoma (HepG-2)**
n-Hexane	10.6	6.9	12.7
EtOAc	60.7	31.5	38.3
n-BuOH	>100	28.6	38.9

Values are presented as mean ± SE of 2 test sample observation, compared with that of control group ($p < 0.05$) for all values.
2 IC50 is defined as the concentration that resulted in a 50% decrease in cell number

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