Research Article

Elemental, antimicrobial and antioxidant activities of a medicinal plant *Sorghum halepense*

Abdul Yasar Khayal¹, Inam Ullah¹, Muhammad Nughman¹, Syed Majid Shah² and Nawab Ali^{1*}

1. Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat-Pakistan

2. Department of Pharmacy, Kohat University of Science and Technology, Kohat-Pakistan

*Corresponding author's email: nawabali_1857@yahoo.com

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Abstract

Sorghum halepense (L.) Pers belongs to the family Graminae or Poaceae, known as Grass family and is an important medicinal plant. It is used as fodder for animals. Present study is designated to find out medicinal properties of this plant. For this purpose the plant samples were collected and acid digested to evaluate the concentration of heavy metals such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) using atomic absorption spectrophotometer. Presence of heavy metals is important for medicinal plants. Then antibacterial, antifungal and antioxidant activities in *Sorghum halepense* was evaluated by macerating and shed drying the plant sample for fifteen days and crude extract, ethyl acetate, n-hexane, chloroform and aqueous fractions were extracted. After careful analysis the concentration of heavy metals were determined as high except Cd and Cr. The crude extract and other fractions of *sorghum halepense* showed good antibacterial activities against *P. aeruginosa*, *S. epidermidis*, *Escherichia coli*, *K. pneumonia* and *B. subtilis* but some of the fractions were not active against some of the bacterial strains. All the fractions showed antifungal activity against *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* except ethyl acetate and aqueous fraction. The ethyl acetate, n-hexane and aqueous fractions showed excellent 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity but crude extract and chloroform didn't show this activity. This plant has shown various positive medicinal attributes and could be used for further bioassays and for isolation of safe herbal drugs.

Keywords: Acid digestion; Crude extract; Heavy metals; Herbal plants; Resistance; Weed

Introduction

For the treatment of various diseases medicinal plants are used globally and these plants are also important in pharmaceutical industries as raw material. These plants show medicinal properties due to the presence of

bioactive compounds and there antimicrobial, analgesic, enzyme inhibition and antioxidant activities, that has various important health effects like discovery of novel drugs, diagnosis and treatment of many diseases [1, 2]. The resistance of microbial strains against

antibiotics is a serious problem throughout the globe. Pharmaceutical industries are struggling very hard in search for new antimicrobials to overcome the challenge of resistance [3]. One possible source for the discovery of new antimicrobials to overcome this challenge of microbial resistance could be plants and hence plants are continually studied for this purpose [4]. World Health Organization has estimated that four billion people around the world uses herbal medicines for their health care [5]. This study is designed to investigate for the first time the elemental analysis, antimicrobial and antioxidant activities of *sorghum halepense*.

Sorghum halepense as shown in figure 1, also known as Johnson grass. It belong to Gramineae family, is an herbaceous perennial plant and is a weed crop found in dry areas, ditches, cultivated fields and waste lands. *Sorghum halepense* has a wide range of uses such as food, beer brewing and livestock feeds and fodder. Their grain is used in biscuits, unleavened bread and tortilla for backing in confectionary industries [6].

The presence of heavy metals and other trace elements are an important criteria for a plant to be medicinal because these elements

help in treatment of various diseases because of their restorative and preventive role. This plant has an amount of heavy metals and other trace elements [7]. So it is necessary to detect levels of various heavy metals and other trace elements in medicinal plants. *Sorghum halepense* is promoted by Wattoo FH *et al.* for treatment of various conditions as a family medicine [8]. This plant also has allelopathic potential that is well documented and this property is supposed to having antimicrobial potential dew to the presence of p-hydroxybenzoic acid, triclin, salcolon and luteolin [9]. A researcher studied that this plant is allelopathic to many crop and weeds [10]. Antioxidants help in protecting the body during oxidative stress generating valuable health effects [11]. Plants and their metabolites are studied to obtain medication by isolation of different chemicals that has therapeutic properties for the cure of many different diseases [12]. Therefore in *sorghum halepense* the amount of many heavy metals, antimicrobial and antioxidant activities were analyzed to make sure whether this plant could be used as medicinal plant or not and we have obtained some positive results.

Figure 1. *Sorghum halepense*
Materials and methods

Plant collection

Plant samples were collected from Kohat during November 2014 in order to analyze the concentration of heavy metals and to find out its antimicrobial and antioxidant activities.

Acid digestion

For heavy metals determination the plant samples were crushed lightly and sieved to pass through 2-mm mesh. In conical flask the sieved sample was treated with 65% nitric acid (HNO₃) for 24 hours at room temperature, then 5ml 70% perchloric acid (HClO₄) was added to it and covered with watch glasses. Contents of the flask were heated on hot plate until the volume of contents in the flask reduces to 1ml. Then

the contents of the flask were cooled, diluted with distilled water and were filtered through whatman filter paper into 25ml volumetric flask and were diluted to the require mark.

Heavy metals determination

Heavy metals like Fe, Cr, Mn, Zn, Pb, Ni, Cu and Cd were estimated with flame atomic absorption spectrophotometer. Calibration standards 0.6, 1.2, 2.4, and 4.8 were arranged from 1000ppm standard stock solution using dilution formula " $C_1V_1=C_2V_2$ " for each heavy metal. Before each analysis the instrument was calibrated. Table 1 shows different measurement condition for each metal under study.

Table 1. Conditions for the measuring of heavy metals

Metals	Mode	Wavelength	Slit width	Current
Fe	Absorption	248.33 nm	1.8/1.35 nm	30 mA
Zn	Absorption	213.86 nm	1.8/1.35 nm	15 mA
Ni	Absorption	232.0 nm	1.8/1.35 nm	25 mA
Cu	Absorption	324.75 nm	2.7/0.8 nm	15 mA
Cr	Absorption	357.9 nm	2.7/0.8 nm	25 mA
Cd	Absorption	228.8 nm	2.7/1.35 nm	4 mA
Mg	Absorption	279.48 nm	1.8/0.6 nm	20mA
Pb	Absorption	283.31 nm	2.7/1.05 nm	10mA

Preparation of crude extract

The plant material was dried in shade and then crushed roughly into powder for the preparation of crude extract. And then softened in methanol with irregular shaking at room temperature for 15 days and then filtered and in rotatory evaporator the filtrate was concentrated at 40°C. The crude extract was suspended in 500 ml distilled water and one after the other separated with n-hexane (3 x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500 ml) to get n-hexane, chloroform and ethyl acetate fractions respectively.

Antibacterial assays

For the determination of antibacterial activity well assay was used. The bacterial strains that were used are *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus epidermidis* and *Staphylococcus Aureus*. Under aseptic conditions the prepared Muller Hinton agar media was transferred into petri dishes and allowed to harden. The altered method of Das *et al.* was followed [13]. Bacterial culture equivalent to 10⁶ cfu was inoculated on hardened media. Metallic borer was used for the formation of wells in media. 200 ul of Dimethyl sulfoxide (DMSO) at concentration of 10 mg/ml of the stock solution of crude extract and fractions were added in the wells. The zones made by the bacterial strains after

24 hours of incubation at 37° C were compared with zone made by the doxycycline that was used as standard drug.

Antifungal assay

For antifungal activity agar well dilution method was used. Three fungal strains *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* were used. In DMSO four dilutions of crude extract and fractions were made i.e. 1.5 mg/ml, 2.5 mg/ml, 3.5 mg/ml and 4.5 mg/ml. In sterile test tubes these fractions were poured that was combined in sea bouard dextrose agar media at 45° C. In each tube a small piece of already grown fungus was placed and incubated for 5 days at 25° C. Results were observed and recorded for each tube. Positive and negative control were included with only fungus and without any fungus respectively.

Antioxidant activity

For determination of antioxidant activity Blois (1958) method with slight modifications was followed [14]. Concisely, the DPPH radical solution of 1mM in methanol was formulated and from this solution 1ml was added to 3ml of sample solutions in ethanol and to control having no sample. After 30 min at 517 nm the absorbance was measured. In results DPPH absorbance will be inversely proportional to DPPH radical-scavenging activity. Free radicals scavenging was calculated by the following formula;

$$\%RSA = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration of extract showing 50% inhibition (EC₅₀) was calculated from the graph of % RSA against the concentration of extract with ascorbic acid, quercetin, α -tocopherol and gallic acid that were used as standards.

Results and discussion

Elemental analysis

The results shows that the concentration of some heavy metals were high like that of Mn from 306±11 $\mu\text{g Kg}^{-1}$, Ni from 81±6 $\mu\text{g Kg}^{-1}$, Cr was 120±8 $\mu\text{g Kg}^{-1}$, Fe was 18±1.8 $\mu\text{g Kg}^{-1}$, and Zn range was 191±6 $\mu\text{g Kg}^{-1}$ while others are present in relatively low amount like Pb from 11±2 $\mu\text{g Kg}^{-1}$, Cu from 2.24±0.6 $\mu\text{g Kg}^{-1}$ and Cd from 1.1±0.1 $\mu\text{g Kg}^{-1}$ as shown in figure 2. The high concentration of some heavy metals depends on the physiographical conditions and waste generated from the area [15]. A researcher reported the concentration of some of the heavy metals in Chinese herbal plants and has found the concentration of, as we have observed of some elements as high while others in low amount [16]. Findings of our work is also in line with the work of a reporter who determined micro and heavy metals accumulation in aloe vera plant [17].

Figure 2. Concentration of heavy metals in *Sorghum halepense*

Antibacterial activity

The antibacterial activity of *Sorghum halepense* extracts were determined by measuring zones of inhibition. The activity of crude extract, fractions and standard are shown in table 2 and figure 3. The crude extract has shown activity against all the bacterial strains. The ethyl acetate and n-hexane fractions were active against *P. aeruginosa* and *S. epidermidis* but didn't against *B. subtilus*, *K. pneumoniae* and *E. coli* while chloroform fraction was active against *S. epidermidis*, *P. aeruginosa* and *B. subtilus*. In this study the plant extracts were active against *Bacillus subtilis* and other bacterial strains. A similar

antibacterial activity was also reported in *Caesalpinia Bandcella* in which they have tested antibacterial activity of *Caesalpinia Bandcella* and our results are in consistency with them [18]. The aqueous fraction of *Sorghum halepense* was active against all the strains except *B. subtilus*. Doxycycline that was used as standard give positive results against all five bacterial strains. Our results are similar to the results obtained by a reporter from South Korea who has reported antibacterial activity of *Sorghum*. They isolated different chemicals and screened them for antimicrobial activity and obtained positive results [19].

Table 2. Antibacterial activity *Sorghum halepense* crude extract and fractions

Micro-org	Zone of Inhibition (mm)					
	Ethyl act	Chloroform	Aqueous	Crude	N-hexane	Doxycycline
<i>E. coli</i>	0	0	12	10	0	16
<i>P. aeruginosa</i>	9	12	9	15	10	19
<i>K. pneumoniae</i>	0	0	12	10	0	24
<i>S. epidermidis</i>	20	14	14	15	19	20
<i>B. subtilus</i>	0	10	0	13	0	17

Figure 3. Antibacterial activity of *sorghum halepense* extracts against different bacterial strains

Antifungal activity

The crude extract and fractions of the plant have shown positive antifungal activity. The Crude extract, n-hexane fraction and

chloroform fraction have shown activity against the three fungal strains used i.e. *A. niger*, *A. fumigatus* and *A. flavus* while ethyl acetate and Aqueous were inactive as shown

in the table 3 and figure 4. For All the three fungal strains, minimum inhibitory concentration (MIC) were 2.5 mg/ml for chloroform and n-Hexane fractions while MIC for crude extract for *A. niger* was 2.5 mg/ml and against *A. fumigatus* and *A. flavus* were 3.5 mg/ml. Several plants have been tested that show antifungal activities [20]. As results obtained by a reporter for some medicinal plants that has shown strong activity against *Aspergillus niger* from Mexico [21]. Tannic acid plays an important

role in antimicrobial activity of medicinal plants and in sorghum it may be due to the presence of this compound. Some phenolics are also thought to contribute in antimicrobial activity. Sorghum is reported to having these compounds [22]. Our results are consistent with the antimicrobial activities revealed by other species of the genus *Sorghum* but not of the *sorghum halepense* as we didn't find any literature on the antibacterial or antifungal activities of *sorghum halepense*.

Table 3. MIC of crude extract and fractions of *Sorghum halepense* against fungal strains

Fungal strains	Ethyle act	Chloroform	Aqueous	N-hexane	Crude Extract
A. Niger	NA	2.5	0	2.5	2.5
A. Fumigates	NA	2.5	0	2.5	3.5
A. Flavus	NA	2.5	0	2.5	3.5

Figure 4. Antifungal activities of different extracts of *sorghum halepense*

Antioxidant activity

DPPH is a compound with free radical that has been commonly used for the determination of free radical scavenging or antioxidant activity [23]. For this activity plant crude extract and fractions were tested and obtained positive antioxidant results as

shown in table 4 and figure 5. An excellent antioxidant activity were shown by n-hexane, ethyle acetate and aqueous fractions and had an EC₅₀ value of 35.53 ug/ml, 30.34 ug/ml and 20.51ug/ml respectively while chloroform fraction and crude extract were not active for this activity. The antioxidant

activity of a medicinal plant was reported by a researcher who has extracted the aromatic geranyl derivative of the resinous exudates of *H. strigosum* and suggested that this plant has strong antioxidant activity [24]. A reporter

has also reported antioxidant and antimicrobial activities of some *sorghum* cereal plants whose results are in consistency with our results for antioxidant and antimicrobial activities [19].

Table 4. Antioxidant activity and EC₁₀ activity of *Sorghum halepense*.

Test Fractions	%Interaction DPPH 30min	EC ₁₀ (ug/ml)
n-hexane fraction	90.70	35.53
Chloroform fraction	37.09	-
Ethyl acetate fraction	94.5	30.34
Aqueous fraction	30.34	20.51
Crude extract	21.34	-

Figure 5. Antioxidant and EC₅₀ of crude extract and fractions of *Sorghum halepense*

Conclusion

From this study it can be concluded that *Sorghum halepense* is a good absorber of metals as it has high concentrations of heavy metals and also this plant has shown good antimicrobial and antioxidant activities, so could be used as a medicinal plant.

Authors' contributions

Designed and performed the experiments: AY Khayal, I Ullah & N Ali, Analyzed the data and contributed the reagents: N Ali, I Ullah, SM Shah & M Nughman, Equally contributed as first author: AY Khayal & I Ullah, Wrote the manuscript: AY Khayal, I Ullah & N Ali.

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