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PROGNOSTICATING THE POTENTIAL OF *Sorghum bicolor* ROOT EXUDATES IN RESPONSE TO ABIOTIC STRESS

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Plant roots represent highly unexplored biological frontier. Roots metabolically produce remarkably diverse group of bioactive compounds which functions as phytoalexins (parasite growth inhibitor), phytotoxins (toxic for plant growth), phytoanticipins (low molecular weight antimicrobial compounds) and germination inhibitors etc. In the present study 2-week-old *Sorghum bicolor* seedlings were transferred to ¼ strength Hoagland solution. After one-week culturing in hydroponic system seedlings were subjected to various levels of abiotic stress (ultraviolet, ultrasound and heat) in combination with permeabilization (to make cell wall perforable) using Tween 20. The exudates were collected after 48 hours of stress exposure. Collected root exudates were evaluated for their phenolic contents and antioxidant potential. *Sorghum bicolor* exposed to different levels of abiotic stress showed a substantial release of bioactive compounds which exhibit significant antioxidant potential. The study revealed different stress levels and exposure time affect root exudation exhibiting maximal exudation after ultraviolet (UV) exposure (136.02±2.01μg / 90.0mL), followed by ultrasound (65.26 ± 1.49 μg / 90.0mL) and heat stress (50.12±0.85 μg / 90.0mL). This study provides an amazing contribution of root exudation as bioactive source and making their fruitful use for various purposes like pharmaceutical applications.
Keywords: *Sorghum bicolor*, abiotic stress, root exudates (RE), antioxidant activity.

INTRODUCTION

Exudates are the secondary metabolites excreted by plant organs such as roots, rhizomes, leaves, stems, and seeds. Exudates include a wide range of compounds like sugars, inorganic ions, amino acids, phenolics, polysaccharides, proteins and enzymes (Muscolo *et al*., 2005; Bais *et al*., 2006). These compounds enter in the environment through various routes like phytovolatilization, leaching and decomposition of plant parts. They have vigorous biological significance as phytoalexins, phytotoxins, phytoanticipins, antimicrobial and signalling agents (Deng *et al*., 2004), germination stimulants (Yoneyama *et al*., 2008) and some of these provide nutrition to soil microbes (Meepagala *et al*., 2005).

Roots are being considered as chemical factories because of synthesis and release of a bewildering variety of exudates. It is being understood that except chlorophyll and few photosynthesises related pigments almost every major plant compound is exuded by plant roots. In addition to other metabolites released by the roots of the plants polyphenols are most common (Muscolo *et al*., 2005; Mandal *et al*., 2009). They have diverse functions like caffeic acid and chlorogenic acid released from watermelon roots to provide resistance toward *Fusarium oxysporum* (Ling *et al*., 2013), canavanine excreted from the roots of leguminous plants help legumes for the attraction of useful soil microbes (Cai *et al*., 2009). Therefore, study of root exudates is an emerging field of interest for developing sustainable management practices and agricultural products like biopesticides and, bioherbicides etc.

Stimulation of exudate synthesis and their release by applying external stress is one of the few strategies that is currently finding commercial application. Abiotic or biotic stress factors trigger increased production of pigments, flavonoid (flavones), phytoalexins, and other defense related compounds (Uddin *et al*., 2010). The use of ultraviolet (UV) radiation as an effective abiotic stress to promote the synthesis of secondary metabolite from *in vitro* cultivated plant cell and tissue cultures has been extensively studied (Afreen *et al*., 2005; Ramani and Chelliah, 2007; Zu *et al*., 2010). Other study like Yang *et al*., (2007) reported that exposure to low dose of UV boosts up the antioxidant pool of winter wheat up to two folds.

Use of external stress has been found to induce general as well as targeted secretion of bioactives like anticarcinogenic glucosinolates in the root exudates of *Brassica rapa* in response to salicylic acid (SA) and methyl jasmonate (MJ) stress. Stress factors not only led to an accumulation of individual compounds in specific organs but also in root exudates, which indicates an extended systematic stress response not only in aboveground plant organs but also in belowground root system and root exudates (Schreiner, 2011). Exploiting this secretion process would allow health

promoting metabolites to be more easily isolated compared to extraction from plant tissues by solvents. However, only limited information is available about how to elicit the production of secondary plant metabolites in root exudates (Dardanelli *et al*., 2010).

Sorghum bicolor (L.) Moench (https://wcsp.science.kew.org/namedetail.do?name_id=443283) is the 5th most important crop and among one of the cereals which are richest in antioxidant compounds. The roots of *S. bicolor* naturally exude a potent phenolic compound known as "sorgolene" which exhibit strong weedicide potential even if present at low concentration of about 10 μM (Hoekenga *et al*., 2003). Some other exudates of sorghum roots are alkyl resorcinol analogues which are accounted for their antifungal properties (Cook *et al*., 2010).

The current research work was designed to investigate the antioxidant potential of sorghum root exudates under controlled conditions of applied stress. To the best of our knowledge, no literature is yet available regarding the antioxidant profile of sorghum root exudates. Therefore, to develop an efficacious plant-based exudation system using optimizing conditions for the production and isolation of bioactives in root exudates, our aims were (1) to assess whether using root exudates as a source of phenolic bioactive is possible (2) to examine the effect of ultraviolet, ultrasound and heat stress on phenolic exudation (3) to find optimal stress factor, stress level and exposure time for phenolic exudation.

MATERIALAS AND METHODS

***Reagents and Standards*:** All potential reagents (Folin-Ciocalteu), standards (DPPH radical, butylatedhydroxytoluene (BHT), trichloroacetic acid, catechin, gallic acid, caffeicacid, vanillic acid, 3,4-dihydroxy benzoic acid, p-hydroxy benzoic acid) and chemicals entities like ammonium thiocyanate, acetic acid, sodium carbonate were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). All the solvents including methanol, ethanol used were of analytical grade and purchased from Merck, Germany.

***Seed collection and Plantation*:** The authenticated and verified seeds of *S. bicolor* were obtained from the Department of Plant Breeding and Genetic (PBG), University of Agriculture, Faisalabad. The seeds were surface sterilized by shaking for 3 min in 96% ethanol, kept in 3% sodium hypochlorite solution for 10 min, rinsed twice in sterile distilled water (SDW), and then left to soak in SDW for 4h at 25°C (Akter, 2016). Surface sterilized seeds were germinated in clear plastic disposable cups (dimensions 100 x 180 mL; 7 seeds/cup), cups were filled with sterile sand and maintained at 25°C in plant growth chamber at Department of Plant Breeding and Genetic (PBG), University of Agriculture, Faisalabad.

***Stress exposure and Exudate collection*:** After 2-weeks (14 DAA, days after appearance) plants were gently rooted out from sand and their roots rinsed carefully with distilled water for 2-3 times to remove sand particles. To enable the roots to grow and flourish well, plants were pre-cultured in nutrient solution for one week (Akter, 2016). For this 2-week old plants were transferred to 50mL transparent glass bottles (82 x 35mm) having 7 seedlings / bottle containing 30 mL of 1/4th strength Hoagland solution to facilitate hydroponic growth conditions. Overall hydroponic system was maintained in a controlled growth chamber at 8h darkness, 60% humidity, 16hr light at 280 μmol photons $m^{-2}s^{-1}$(by fluorescent lamp) and 25°C with permanent aeration of hydroponic system.

After one-week culturing in Hoagland solution the nutrient solution was replaced with ultrapure water. Three transparent glass bottles (82 x 35mm) having 7 seedlings/bottle were exposed to respective stress according to the plan mentioned in Table 1. Root exudates (RE) were collected after 48h stress exposure and stored at –20°C.

Table 1. Treatment plan adopted to study *Sorghum bicolor* root exudation.

UV + Tween 20	Wavelength	Exposure Time
	UV-A (340 nm)	40 min
		80 min
		120 min
	UV-B (290 nm)	40 min
		80 min
		120 min
	UV-A + T2%	40 min
		80 min
		120 min
	UV-B + T2%	40 min
		80 min
		120 min
Ultrasound	**Power/Frequency**	**Exposure Time**
	US1- (13.7 Mw/cm^3 (38kHz))	3min
		6min
		9min
	US2-(61.4 Mw/cm^3 (38kHz))	3min
		6min
		9 min
	US1-T2%	3min
		6min
		9 min
	US2-T2%	3min
		6min
		9 min
Heat	**Temperature**	
	H1-36 ^{0}C	4 h
		8 h
	H2-40 ^{0}C	4 h
		8 h
	H1-T2%	4 h
		8 h
	H2-T2%	4 h

	8 h
	@ Relative Humidity 65% measured by pyschrometer

***Assessment of in vitro Antioxidant Activity of Exudates*:** The antioxidant activity of the exudates was measured (*in-vitro* antioxidant models) by performing following group of analysis:

***Assessment of TPC in Sorghum Root Exudate (SRE)*:** For the assessment of TPC (total phenolic contents) of sorghum root exudate (SRE) well reported method was adopted (Chaovanalikit and Wrolstad, 2004). Amount of total phenolic contents were assessed by using Folin-Ciocalteu reagent. In short crude RE (50mg) was vortex with 0.5 mL of FC (Folin-Ciocalteu reagent) and 7.5 mL of distilled H_2O. After incubating the mixture at room temperature for 10 min, 1.5 mL of sodium carbonate was added to it. The mixture again incubated over a water bath for 20 min at 40℃ and cooled in an ice bath. A spectrophotometer (U-2001, Hitachi Instrument Inc.Tokyo, Japan) was used to take absorbance at 755 nm. Total amount of phenolic contents was measured from calibration curve which was constructed using gallic acid with concentration range of 2-200 ppm (R^2 =0.9952). Sample analysis were carried out thrice and results were averaged.

***Determination of radical scavenging capacity*:** Radical scavenging capacity of Sorghum RE was determined spectrophotometrically by a well reported method (Iqbal and Bhanger, 2007). Sorghum RE was diluted with methanol at six different concentrations. In a dry test tube, 1.0 mL of each concentration was mixed with 5.0 mL of DPPH. After incubation at room temperature for 30 minutes' absorbance of test solution was taken at 517 nm. Percentage inhibition of DPPH was calculated using following equation;

Scavenging capacity = (1-absorbance sample / absorbance control) 100

The results were expressed in IC_{50} value (μg/mL), which is the plot of concentration against percentage inhibition.

***Assessment of reducing power*:** Reducing power of Sorghum RE was determined at four different concentrations (2.5, 5.0, 7.5, 10.0 mg /mL) following the method of Sultana *et al*., (2009). Each concentration was added with 5.0 mL of sodium phosphate buffer and 5.0 mL of $[K_3Fe(CN)_6]$. After incubating the mixture at 50^0C for 20 minutes trichloroacetic acid (5.0 mL, 10%w/v) was added and whole mixture was centrifuged at 3000 rpm for 10 min. After centrifugation upper layer was separated out up to 5.0 mL and 5.0 mL of distilled water along with 1.0 mL of $FeCl_3$ (0.1%w/v) was added to it. The absorbance was taken at 700 nm. Vitamin C was used as positive control.

***Assessment of Total Flavonoid contents (TFC) in Sorghum RE*:** The flavonoid contents of Sorghum RE were assessed by a method of Dewanto *et al*., (2002) with slight modification. Shortly 1.0 mL of RE having concentration 0.1 mg/mL was diluted with 4.0 mL of distilled H_2O and mixed with 0.3 mL of $NaNO_2$ (5%). After 5 min0.3 mL of $AlCl_3$ (10%) was added followed by 2.0 mL of NaOH (1.0 M) after another 5min. The mixture was then insipid with 2.4 mL of distilled H_2O and shake vigorously. Test absorbance was measured at 510 nm. TFC were expressed as Catechin equivalents (μg CE/g RE).

***Test Statistics*:** All analyses were performed thrice and expressed as mean± S.D. Analysis of variance was performed using ANOVA. Student's t-test was applied for comparison of the means. $p< 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

***Ultraviolet treatment*:** Ultraviolet radiation has been well studied as an effective abiotic stress to promote the biosynthesis of bioactive compounds. The target site of UV radiation is amino acids, quininones, membranes, pigments, photosynthetic machinery and UV absorbing aromatic chemical groups (Vass *et al*., 2005; Zu *et al*., 2010). The discrepancy in the yield of SRE in response to UV stress for varying duration has been listed in Table 2. Generally, the yield of sorghum root exudates (SRE) varied from 26.60 ± 0.52 to 136.02 ± 2.10 μg / 90.0 mL RE. The results showed that exposure to UVB (290 nm) stress for 80 min and then permeabilizing with Tween 20 (2%) gave significantly higher yield (136.02 ± 2.10 μg / 90.0 mL RE) as compared to both control groups. This might be considered due to combined effect of UV and Tween 20 (Gontier *et al*., 2002). Tween 20 increased the excretion by permeabilizing cell membrane and UV is found an effective elicitor to promote the synthesis of bioactive compounds (Ramani *et al*., 2007). The lowest yield (26.60 ± 0.52 μg/90mL RE) was obtained with UVA (340 nm) for longer exposure time (120 min) in the absence of any permeabilization treatment. It has been observed that plants when exposed to UV for longer periods of time results in the loss of plant vitality which in turn cause the poor yield of bioactives. UV exposure for longer time also results into negative impact on photosynthesis related parameters like thylakoids and grana which are very sensitive to UV radiations (Baroniya *et al*., 2014). The discrepancy in the phenolic contents of sorghum RE in response to ultraviolet stress are listed in Table 2. Overall TPC varied from 3.85 ± 0.06 to 42.38 ± 1.22 μg gallic acid equivalent / 90mL RE. Lowest one drained from water control (3.85 ± 0.06 μg GAE/90.0mL RE) in which no stress was applied, while hefty level (42.38 ± 1.22 μg GAE/90.0mL RE) was found to be liberated from UVB + T20 treatment. Such significant difference in phenolic contents validates the synergistic effect of UVB and Tween 20 on amplified release of bioactives. Ultraviolet stress was also found to enhance the ascorbate levels of spinach up to 2.7fold, antioxidant activity of lettuce was also found to be enhanced with substantial increased of tocopherol levels by

Table 2. Yield, total phenolic contents, DPPH IC_{50} and total flavonoid contents of *Sorghum bicolor* root exudates in response to Ultraviolet stress.

Sr. #	Treatment	Exposure time	Yield *μ*g/90Ml	TPC *μ*g GAE/90mL	TFC *μ*g CE/90mL	IC_{50} *μ*g /90mL	ABTS mM TE/90mL
T1	UV-A(340)	40	43.29±0.54Bf	11.59±0.33Bf	6.18±0.12Bg	28.06±0.85Ac	7.09±0.12Bh
T2		80	63.31±0.89Ae	17.94±0.17Ae	7.58±0.08Af	25.89±0.88Bc	11.43±0.14Ac
T3		120	26.60±0.52Ch	9.13±0.26Cg	4.03±0.03Ci	33.63±0.62Cb	6.31±0.17Bh
T4	UV-B(290)	40	68.30±1.36Be	16.61±0.33Be	9.39±0.14Be	17.79±0.24Be	7.31±0.18Cgh
T5		80	96.59±2.86Ad	26.46±0.52Ac	10.44±0.21Ad	13.37±0.21Cf	9.75±0.17Ade
T6		120	33.60±0.51Cgh	8.04±0.18Cg	5.37±0.09Ch	21.88±0.42Ad	8.12±0.17Bfg
T7	UV-A+T 2%	40	107.11±1.91Bc	21.71±0.48Bd	9.30±0.18Be	9.10±0.06Bhi	9.09±0.16Cef
T8		80	123.16±2.94Ab	35.14±0.53Ab	12.09±0.09Ac	7.64±0.18Chi	15.93±0.22Ab
T9		120	30.00±0.72Ch	13.66±0.14Cf	4.59±0.07Ci	11.88±0.31Afg	10.19±0.02Bd
T10	UV-B+T2%	40	124.39±2.13Bb	27.77±0.29Bc	14.98±0.12Bb	7.37±0.13Bi	10.44±0.29Bcd
T11		80	136.02±2.10Aa	42.38±1.22Aa	18.18±0.25Aa	2.93±0.05Cj	18.95±0.39Aa
T12		120	88.31±1.66Cd	7.92±0.22Cg	4.63±0.08Ci	9.90±0.13Agh	8.68±0.25Cf
T13	Control-1	T 2%	41.61±0.63fg	7.28±0.09g	2.56±0.04j	32.98±0.43b	8.53±0.10f
T14	Control-2	Water	7.08±0.14i	3.85±0.06h	1.62±0.02k	60.29±0.57a	2.07±0.03i

Means sharing similar letter in a column are statistically non-significant ($p>0.05$). Small alphabets show the significance among applied stress while capital alphabets represent the significance level among exposure time.

Table 3. Reducing power of *Sorghum bicolor* root exudates in response to Ultraviolet stress

Stress	Exposure Time (min)	Reducing power at λ=700nm			
		2.5*μ*g/90.0mL	5*μ*g/90.0Ml	7.5*μ*g/90.0mL	10*μ*g/90.0 mL
UV-A	40	0.25±0.02	0.31±0.03	0.34±0.02	0.42±0.02
	80	0.44±0.03	0.47±0.02	0.54±0.03	0.54±0.02
	120	0.21±0.03	0.24±0.02	0.34±0.03	0.43±0.01
UV-B	40	0.33±0.02	0.42±0.02	0.46±0.02	0.50±0.02
	80	0.39±0.01	0.50±0.02	0.51±0.02	0.57±0.02
	120	0.19±0.02	0.24±0.03	0.32±0.02	0.37±0.03
UV-A + T 2%	40	0.30±0.05	0.32±0.04	0.35±0.04	0.41±0.02
	80	0.46±0.02	0.53±0.02	0.58±0.02	0.71±0.02
	120	0.29±0.03	0.39±0.03	0.43±0.02	0.46±0.02
UV-B + T2%	40	0.41±0.06	0.54±0.03	0.55±0.03	0.57±0.06
	80	0.63±0.02	0.66±0.01	0.74±0.02	0.84±0.04
	120	0.24±0.02	0.31±0.02	0.37±0.02	0.42±0.02
Control-positive	T 2%	0.28±0.03	0.29±0.04	0.34±0.03	0.39±0.03
Control-negative	Water	0.15±0.06	0.20±0.03	0.23±0.02	0.27±0.01

Values are means± SD of triplicate for each treatment. Statistical significance was checked at 95% of CI ($p < 0.05$).

treating lettuce with UVB for one week (Hueberger *et al*., 2004; Hideg *et al*., 2013). Radical scavenging potential of sorghum DRE varied 60.29±0.57 - 2.93±0.57 *μ*g/90.0mL DPPH IC_{50} in response to ultraviolet stress (Table 2). UVB + Tween 2% liberated exudates after the exposure time of 80 min were found more effective scavengers of DPPH radicals exhibiting lower IC_{50} (2.93±0.57 *μ*g/90.0 mL) among the other treatments.

In context to concentration of total flavonoids the maximum R amount of total flavonoid contents (TFC) 18.18 ± 0.25 *μ*g CE/90mL RE (Table 2) were found in exudates released from UVB + T20 treated sorghum after the exposure time of 80 min. Our results are in agreement with some other research findings showing that UV-C treated Blueberry, Broccoli and Tomato have increased phenolic and flavonoid contents (Perkins-Veazie *et al*., 2008). The increase in the phenolic and flavonoid contents is due to the activation of phenylalanine ammonia lyase (PAL) and chalcone synthase by UV irradiation that is responsible for their biosynthesis in plant tissues (Alothman *et al*., 2009; Papoutsis *et al*., 2016). The reducing potential of sorghum RE for different treatments of UV stress and Tween 20 permeabilization is listed in Table 3. The reducing potential at 10 *μ*g / 90.0mL varied 0.84 ± 0.04 -0.37 ± 0.03. The absorbance of the tested exudate varied linearly with exudate concentration (2.5–10.0 *μ*g / 90.0mL). The highest reducing potential (0.84 ± 0.04) was observed for UVB+T20 after 80 min of UV exposure whereas lowest (0.37 ± 0.03) was resulted from UVB at 120

min of stress revelation at the concentration of 10.0 μg/90.0 mL. Data regarding TEAC presented in Table 2. indicated that sorghum root exudates exhibit appreciable antioxidant capacity equivalent to 8.53 ± 0.1–18.95 ± 0.39 mM TE / 90.0mL. Overall, UV stress liberated exudates have better ABTS radical scavenging as compared to control. Among different stress levels and exposure times, T11 (UVB-T20, 80 min) shows highest TEAC value 18.95 ± 0.39 mM TE/90.0 mL RE.

***Ultrasound treatment*:** Low energy ultrasound (US) is an effective, non-invasive and potent abiotic stress which induces defense responses in plants and stimulates the production of secondary metabolites. One of the most remarkable feature of US in biological systems is cell permeabilization caused by microstreaming of US. This effect may be useful for harvesting and release of intracellular products, such as secondary metabolites from cultured plant cells and living plants. Microstreaming causes shear stress and enhance mass transfer inside and outside of the cell to stimulate metabolic activity (Lin *et al*., 2001). Sorghum RE maintained virtuous polyphenolic contents at all treatments of ultrasound stress and expressed in Table 4. The maximum TPC were expressed by T10 (13.75 ± 0.18 μg GAE/90.0mL) while lowest were expressed by T14 (1.13 ± 0.02 μg GAE/90.0mL). Data regarding TEAC presented in Table 4 indicated that sorghum root exudates exhibit

Table 4. Yield, total phenolic contents, DPPH IC_{50} and total flavonoid contents of *Sorghum bicolor* root exudates in response to Ultrasound stress.

Sr.	Treatment	Exposure time	Yield μg/90.0mL	TPC μgGAE/90.0mL	TFC μg CE/90.0mL	IC_{50} μg /90.0 mL	ABTS mMTE/90.0mL
T1	US-1	3 (25.5mW/cm²)	32.31±0.57Ae	10.25±0.20Ac	5.65±0.11Ab	48.09±1.55Chi	35.23±0.73Ac
T2		6	22.29±0.36Bg	6.27±0.18Bf	3.13±0.06Bf	62.76±0.93Bef	25.18±0.34Bde
T3		9	21.89±0.44Bg	6.43±0.19Bf	3.12±0.03Bf	88.22±1.38Ac	16.56±0.33Cf
T4	US-2	3 (40.3mW/cm²)	46.35±0.57Ac	11.73±0.28Ab	6.99±0.09Aa	51.41±1.05Cgh	38.99±0.57Ac
T5		6	39.62±0.76Bd	8.43±0.15Bde	3.81±0.10Be	65.26±0.73Bde	35.50±1.06Ac
T6		9	26.25±0.59Cf	7.56±0.05Ce	3.11±0.03Bf	71.09±1.64Ad	15.81±0.29Bf
T7	US1-T2%	3	52.59±1.37Ab	12.01±0.40Ab	5.74±0.04Ab	41.32±0.37Ci	43.29±1.36Ab
T8		6	33.41±0.53Be	9.41±0.18Bcd	4.65±0.08Bd	58.12±1.24Bfg	24.71±0.60Bde
T9		9	20.84±0.36Cg	8.86±0.17Bd	2.88±0.05Cf	61.87±0.85Aef	15.51±0.12Cf
T10	US2-T2%	3	65.26±1.49Aa	13.75±0.18Aa	5.29±0.13Ac	27.87±0.23Cj	58.72±1.40Aa
T11		6	37.25±0.90Bd	11.53±0.27Bb	4.51±0.03Bd	98.67±1.18Ab	28.07±0.84Bd
T12		9	22.68±0.17Cfg	8.37±0.20Cde	4.02±0.03Ce	88.11±1.30Bc	22.09±0.56Ce
T13	Control-positive	T 2%	9.03±0.21h	3.21±0.06g	1.75±0.05g	91.43±2.96c	10.28±0.21g
T14	Control-negative	Water	6.02±0.18h	1.13±0.02h	0.81±0.02h	111.51±0.85a	5.67±0.16h

Means sharing similar letter in a column are statistically non-significant (p>0.05). Small alphabets show the significance among applied stress while capital alphabets represent the significance level among exposure time.

Table 5. Reducing power of *Sorghum bicolor* root exudates in response to Ultrasound stress.

Treatment	Exposure Time (min)	Reducing power at λ=700nm			
		2.5μg/90.0mL	5.0μg/90.0mL	7.5μg/90.0mL	10.0μg/90.0mL
US-1	3	0.711	0.767	0.475	0.036
	6	0.411	0.388	0.436	0.753
	9	0.731	0.723	0.66	0.408
US-2	3	0.441	0.430	0.448	0.459
	6	0.445	0.458	0.441	0.416
	9	0.423	0.432	0.422	0.542
US-1 + T 2%	3	0.634	0.688	0.688	0.626
	6	0.715	0.663	0.694	0.676
	9	0.731	0.768	0.781	0.629
US-2 + T2%	3	0.634	0.688	0.688	1.626
	6	0.715	0.663	0.694	0.676
	9	0.581	0.870	0.185	0.382
Control-positive	T 2%	0.711	0.061	0.440	0.692
Control-negative	Water	0.091	0.147	0.199	0.136

Values are means± SD of triplicate for each treatment. Statistical significance was checked at 95% of CI ($p < 0.05$). DRE represent Dry root exudate.

appreciable antioxidant capacity equivalent to 5.67 ± 0.16 – 58.72 ± 1.40 mM TE/90.0mL. Among different stress levels and exposure times, T10 (US2-T20, 3 min) shows highest TEAC value 58.72 ± 1.40 mM TE/90.0 mL RE. Total flavonoid contents (TFC) of US stressed sorghum root exudates ranged 5.29 ± 0.13 - 0.81 ± 0.02 μg CE/90.0mL RE as shown in Table 4. The IC_{50} value ranged from 111.51 ± 0.85 to 27.87 ± 0.23 μg/90.0mL. Exudates with lower IC_{50}value (27.87 ± 0.23 μg / 90 mL) released from US2-T2% treated sorghum after the stress exposure of 3 min exhibiting higher radical scavenging capacity. This represents the dual effect of ultrasound and tween 20 on exudate release. Reducing power (RP) was determined by ferrous ion chelating assay and presented in Table 5. The highest absorbance at 700nm represents the greater reducing power of the sample. Sorghum RE showed highest reducing power at the concentration of (10μg / 90.0 mL) after the US2 stress exposure of 3min with Tween 20 permeabilization. The lowest reducing power was exhibited by US1 exudates after the US exposure time 9 min which might be due the harmful effects of ultrasound radiations.

***Heat treatment*:** Fortuitously plants have developed various defensive mechanisms under heat stress. Heat stressed plant cells protect themselves by releasing a variety of antioxidant compounds such as ascorbate, glutathione, carotene, and tocopherols which play important role in the removal of toxic oxygen compounds by controlling the intra-cellar ROS content (Liu and Huang, 2000; Fu and Huang, 2001). A study conducted on heat acclimated turfgrass suggested that heat stress causes the over production of ROS (reactive oxygen species) and plants tolerate to ROS by enhanced production of antioxidant metabolites like ascorbate (AsA), glutathione (GSH), tocopherol and carotene etc. A number of studies conducted on heat stressed plants concluded that total antioxidant activity was found maximum at 35 – 40 ^{0}C and then decrease. Polyphenolic contents of sorghum RE at all treatments of heat stress expressed in Table 6 and ranged 3.85 ± 0.12 - 32.87 ± 0.76 μg GAE/90.0 mL RE. From the

Table 6. Yield, total phenolic contents, DPPH IC_{50} and total flavonoid contents of *Sorghum bicolor* root exudates in response to Heat stress.

Sr.	Treatment	Exposure time	Yield μg/90.0 mL	TPC μg GAE/90.0 mL	TFC μg CE/90.0mL	IC_{50} μg /90.0mL	ABTS mM TE/90.0mL
T1	H1(36℃)	4h	37.56±0.87Bc	16.38±0.39Bd	4.54±0.01Bd	53.75±0.81Ad	19.05±0.16Bd
T2		8h	43.21±1.36Ab	27.54±0.61Ab	7.76±0.09Ab	35.94±0.40Be	43.54±0.65Ab
T3	H2(40℃)	4h	21.86±0.61Bf	10.72±0.31Af	3.03±0.08Be	58.21±1.61Abcd	14.94±0.48Ae
T4		8h	27.06±0.35Ae	11.61±0.30Aef	4.39±0.07Ad	57.38±1.74Acd	11.61±0.15Bf
T5	H1+ T 2%	4h	41.78±0.80Bb	21.29±0.38Bc	5.43±0.06Bc	63.62±0.71Ab	23.55±0.58Bc
T6		8h	50.12±0.85Aa	32.87±0.76Aa	9.87±0.06Aa	31.11±0.27Be	51.89±0.92Aa
T7	H2+ T2%	4h	26.34±0.42Be	10.72±0.17Bf	4.58±0.10Ad	61.19±0.61Bbc	17.35±0.38Bde
T8		8h	31.28±0.60Ad	13.39±0.31Ae	4.32±0.08Ad	73.54±2.06Aa	25.05±0.68Ac
T9	Control-positive	T2%	41.61±0.46b	7.28±0.10g	2.56±0.06f	32.98±0.28e	8.53±0.20g
T10	Control-negative	Water	7.08±0.11g	3.85±0.12h	1.62±0.04g	60.29±0.45bc	2.07±0.05h

Means sharing similar letter in a column are statistically non-significant (p>0.05). Small alphabets show the significance among applied stress while capital alphabets represent the significance level among exposure time.

Table 7. Reducing power of *Sorghum bicolor* root exudates in response to Heat stress.

Stress	Exposure Time (h)	Reducing power at λ=700nm			
		2.5μg/90.0mL	**5μ/90.0mL**	**7.5μg/90.0mL**	**10μg/90.0mL**
H1	4h	0.17±0.04	0.59±0.05	0.81±0.06	1.05±0.06
	8h	0.66±0.02	0.89±0.03	1.07±0.03	1.48±0.04
H2	4h	0.91±0.03	0.35±0.04	1.51±0.05	1.71±0.05
	8h	0.17±0.04	0.75±0.05	1.39±0.07	1.81±0.09
H1 + T 2%	4h	0.91±0.03	0.20±0.04	1.71±0.05	1.98±0.06
	8h	0.53±0.02	0.79±0.02	1.07±0.03	2.25±0.04
H2 + T2%	4h	0.61±0.02	0.91±0.03	1.25±0.04	1.46±0.04
	8h	0.75±0.02	0.11±0.03	1.70±0.05	1.79±0.05
Control-positive	T2%	0.80±0.02	0.19±0.04	1.61±0.05	1.91±0.06
Control-negative	Water	0.31±0.03	0.35±0.04	0.51±0.05	0.71±0.05

Values are means± SD of triplicate for each treatment. Statistical significance was checked at 95% of CI (p< 0.05). DRE represent Dry root exudate.

Table 6, it was observed that heat stress significantly induced exudation of polyphenolic compounds. TPC exuded from heat (36 ^{0}C) stress after exposure time of 8h with tween 20 permeabilization were higher as compared to other treatments. Natural exudation method was found superior to other conventional extraction methods because antioxidant activities of plant extracts are affected by harsh extraction conditions of conventional extraction methods (Michiels *et al*., 2012). As shown in Table 6, sorghum root exudates resulted from all treatments of heat stress showed obvious scavenging of DPPH radical. However, exudates liberated from T6 showed higher DPPH radical scavenging activity compared with exudates of other treatments. The reducing power of polyphenolic extract have been regarded as a significant indicator of its potential antioxidant activity and shown in Table 7. As shown from Table.7.the sorghum DRE exhibited significant ($p < 0.05$) reducing power. All treatments of heat stress showed obvious reducing potential of sorghum root exudates in a concentration dependent manner. At a concentration of 10.0μg/mL the reducing potential was found higher for all treatments. Total flavonoid contents of sorghum RE in response to heat stress are expressed in Table 6. The highest amount of flavonoid content was observed from T6 after the heat exposure of (36^{0}C) for 8hr with tween 20 permeabilization.

***Conclusion*:** To address the draw backs of organic solvents in basic extraction and exudates collection, a classical method of exudate collection was adopted in present study. In this method plants are pulled out from soil/sand media and transferred into the aqueous medium for a specific time period to collect the exudates from roots. The collected exudates are then filtered and filtrate is then further subjected to chemical profiling of exudate bioactives and for their prospective potential biological applications. Plant's interaction with stress factors affects the plant metabolite production. Therefore, by customizing the quantity and quality of applied stress, bioactive production can be optimized. The applied stress factors in present study are UV radiation, ultrasound, heat and Tween 20. *S. bicolor* root exudates were studied in response to applied stress. Antioxidant potential of liberated exudates was determined according to standard protocols. Overall research work revealed that among applied levels of stress, UVB in combination with tween 20 gave greater yield than other applied stress followed by US2 with 3min exposure and H1 of 8h exposure.

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