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Phytochemical composition of wild *Sorghum* by GC-MS

Dora S. V. V. S. N.[1], Sarada Mani N[2] , Sharmila Polumahanthi[3]
[1](Department of Botany, Andhra University, India)
[2](Department of Botany, Andhra University, India)
[3](Department of Botany, Andhra University, India)

ABSTRACT : *The study was carried out to determine the phytochemical components of chloroform extracts of wild genotype IS 27703 of Sorghum bicolor, a hardy cereal crop. GC-MS analysis was performed on the tissue culture phenolic exudates after inoculation of the immature inflorescence explant into MS medium and interpretation on mass spectrum was conducted using the database of National Institute of Standard and Technology. 15 compounds were identified. The predominant compound was Dioctyl phthalate (90.21%) with molecular weight 390.5561. The other components were found to be hydrocarbons. Dioctyl phthalate was observed to be useful as a bioplasticizer.*

KEYWORDS : *Cereal, Gas chromatography, Mass spectroscopy, Phytocomponents, Sorghum.*

I. INTRODUCTION

Sorghum is an important cereal and a very good food source in Africa and Asia and is widely grown in the southern United States as a cattle feed. In India, it was grown in 7381700 Ha that yielded 9487 Hg/Ha and recorded as top producer of *Sorghum* in the world. It ranks fifth in India for commodity value [1]. Nigeria was the top producer with 6900000 metric tons production followed by USA and India [2]. Among cereals, *Sorghum* has the highest content of phenolic compounds reaching up to 6% (w/w) in some varieties [3], [4]. *Sorghum* genotypes differ in oligomeric and polymeric compounds that can be analyzed for their components. Sorghum and millet phenols are difficult to identify and characterize due to lack of standards. However, preliminary phytochemical screening of *Sorghum* showed the presence of phenolic compounds, antioxidants, tannins, carotenoids, alkaloids and terpenes. Methods used to structurally characterize these compounds include mass spectrometry, 1H and 13C nuclear magnetic resonance spectrometry and infrared spectroscopy [5]. Nevertheless, plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted secondary metabolites. In many cases, these substances serve as plant defense mechanisms against infection by microorganisms, predation by insects and herbivores. Polymeric polyphenols, and antifungal proteins (AFP) expressed naturally in plants confer resistance to many plant pathogenic fungi. Many such proteins have been identified in different plants and also the phenols are used for developing resistance against fungal diseases [6]. The presence of diverse secondary metabolites has been reported from pigmented lines of Sorghum. However, there has been not much information available on phytochemical components and biological activity in the invitro extracts. The objective of the present study is to identify the phenolic compounds that were released during invitro tissue culture of various explants of three different genotype of sorghum IS 27703 by using GCMS. In Sorghum various phenolic compounds have been reported like polyflavanols [7], anthocyanins [8, 9] phenolic acids [10, 11] tannins [12] and other antioxidant compounds. They are considered to be of neutraceutical importance [13, 14]. High levels of various phenolic compounds have been reported in *Sorghum* [15, 16, and 17]. Gas chromatography-Mass spectroscopy analysis separates all of the components in a given sample and provides a representative spectral output. Each chemical compound has a characteristic mass spectrum. So, we can identify a compound by comparing the unknown compound's mass spectrum with known compound. A mass spectrum will display a peak for the unregimented molecule of that known compound. Moreover, the compounds were separated for a reliable and conclusive identification. The study was designed to determine possible chemical components from the exudations that were released by the explant into the tissue culture MS medium. After 2 weeks of inoculation of the explant, the medium contained more amounts of phenolic secretions and fungal contamination was also not evident. But in the absence of phenolic secretions, fungal contamination was observed. It was then suspected that the absence of fungal contamination was due to the phenolic secretions. We investigated on about the nature of that phenolic compound.

II. MATERIALS AND METHODS

2.1 Plant material: The seeds of genotype of Sorghum IS 27703 were provided by Germplasm unit of International Crop Research ICRISAT, Hyderabad.

2.2 Explant treatment and sample preparation: Immature inflorescences enclosed in the boot leaf were collected from field grown plants, swabbed in 70% ethanol after removing outer whorl of leaves. Small panicles (1-4cm) were aseptically cut open and were surface sterilized in 70% ethanol for a minute and 3% sodium hypochlorite for 10 minutes and then rinsed 10 times with sterile distilled water. This explant was cut into small pieces (0.5cm or less) and then 8-10 explants were inoculated on the petri plate with their bases touching the MS-medium containing 1.5mg/L 2, 4-D + 0.5KN mg/L. on 12^{th} day after inoculation, we observe dark brown and black pigmented exudations around the explant. The explant was carefully removed and the pigmented portions were excised immediately. The excised material was dissolved in 5ml of chloroform for the extract preparation and then filtered. The filtrate was then examined for the possible chemical compositions.

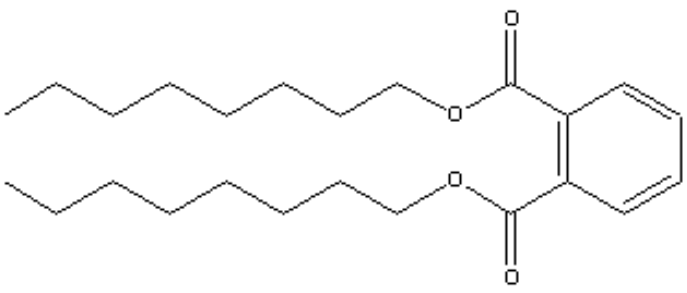
2.3 Gas chromatography and Mass spectroscopy: GC-MS analysis of the immature inflorescence during invitro culture of chloroform extracts of Sorghum genotype IS 27703 was performed using a Shimadzu QP 5050, instrument with a gas chromatograph interfaced to a mass spectrometer (GC-MS), equipped with EI mode with DB 5 capillary column (0.25mm OD x 30 meter). An electron ionization system was operated in electron impact mode with ionization energy of 70eV. Helium gas was used as a carrier gas at a constant flow rate of 1.5ml/min.

III. RESULTS AND DISCUSSION

The investigation was carried out to determine the possible chemical components from $CHCl_3$ extracts of Sorghum bicolor by GC-MS. The analysis gave the GC-MS Chromatogram which indicated a mixture of compounds. The components present in the extract were identified by using standard NIST library and the properties like nature of the compound, molecular formula, retention time, molecular weight and concentration (%) were represented in table 1. The peak of the chromatogram (90.21%), the major component, was identified as Dioctyl phthalate with molecular weight 390.5561. The remaining compounds in the mixture were mostly hydrocarbons (Figure 2) with % concentrations 3.18%, 2.95%, 2.64% and 1.01% respectively. All the compounds varied in their retention times. The amount of the time that a compound retained in the GC column is known as retention time. The relative percentage amount of each compound was calculated by comparing its average peak area to the total areas. The quantitative determination of the chemical compounds was based on the comparison of the peak areas of samples with those in GCMS library. The identification of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns. Interpretation of mass spectrum of GC-MS was conducted by using the database of National institute of standard and technology (NIST) having more than 62,000 patterns. The spectrum of an unknown compound was compared with the spectrum of a known compound. GC-MS chromatogram analysis of the chloroform extract of sorghum showed 5 peaks. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component. The retention time, Molecular formula, Molecular weight and concentration (%) of the tested sample was tabulated and represented in (Table 1). A total of 15 compounds were identified in the chloroform extract of tissue culture medium exudations of Sorghum. 2 unknown compounds were noticed with molecular weight 198 and molecular formula $C_{14}H_{30}$. The spectra revealed the major five compounds present were with the molecular formula $C_{24}H_{38}O_4$.

Chemical Formula of Dioctyl phthalate: $C_{24}H_{38}O_4$

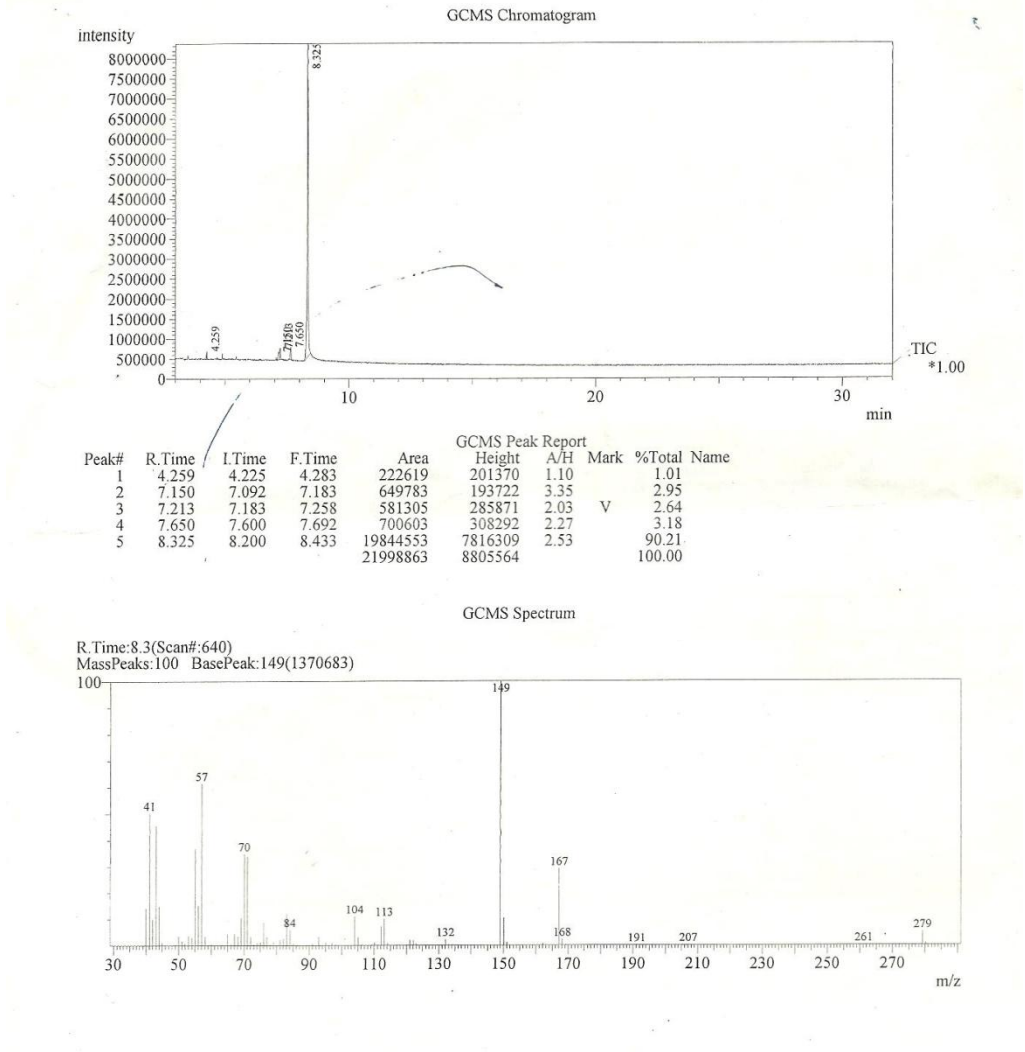
Chemical Structure:



Molecular mass: 390.5561

Sorghum cultivars resistant to fungal attack contained both a greater variety and larger amounts of phenolic acids in the free form. Tannins protect the grain against insects, birds, fungi, and weathering. These beneficial effects ensure that brown sorghums will continue to be produced in certain pest-ridden areas of the world [6]. [18] Partitioned sorghum phenolic acids and concluded that white cultivars without pigmented testa contained the lowest amount of phenolic acids. Brown cultivars contained higher levels of free phenolic acids and compounds and were more resistant to grain weathering. [10] separated, by reverse phase HPLC (high performance liquid chromatography), free and bound phenolic acids of sorghum. Eight main phenolic acids with different polarity were identified in extracts. These included Gallic, Protocatechuic, *p*-Hydroxybenzoic, Vanillic, Caffeic, *p*-Coumaric, Ferulic, Cinnamic acids. Dioctyl phathlate is the major component of the phenolic secretions of the three varieties of sorghum (*Sorghum bicolor* (L.) Moench). Many sorghums with tannins and higher levels of phenol based pigments are resistant to molding; these compounds cause dark colors, astringency, and or decrease nutritional value in foods or feeds [19, 20]. [21] analyzed fatty acid composition of seed oil of different varieties of *Sorghum bicolor*. They applied GC-MS for analysis of oil components. They reported higher PUFA than MUFA and specified SFA's like octanedecoic acid and azelaic acid. An elaborative study was done by [22] on application of GC-MS for the detection of lipophillic compounds. They studied about lipophillic metabolite profiles in roots and shoots of agar-grown seedlings of rsr g-1 Arabidopsis mutant, green and red cuticles of DFD mutant of tomato and Alisa Craig wild type tomato. [23] analyzed hydrophobic root exudates of Sorghum and implications on the parasitic plant *Striga asiatica* by GC-MS.

FIGURES AND TABLES



GCMS Peak Report

Peak#	R.Time	I.Time	F.Time	Area	Height	A/H	Mark	%Total	Name
1	4.259	4.225	4.283	222619	201370	1.10		1.01	
2	7.150	7.092	7.183	649783	193722	3.35		2.95	
3	7.213	7.183	7.258	581305	285871	2.03	V	2.64	
4	7.650	7.600	7.692	700603	308292	2.27		3.18	
5	8.325	8.200	8.433	19844553	7816309	2.53		90.21	
				21998863	8805564			100.00	

Fig 1. GCMS Chromatogram and Spectrum of *Sorghum* tissue culture phenolic exudates.

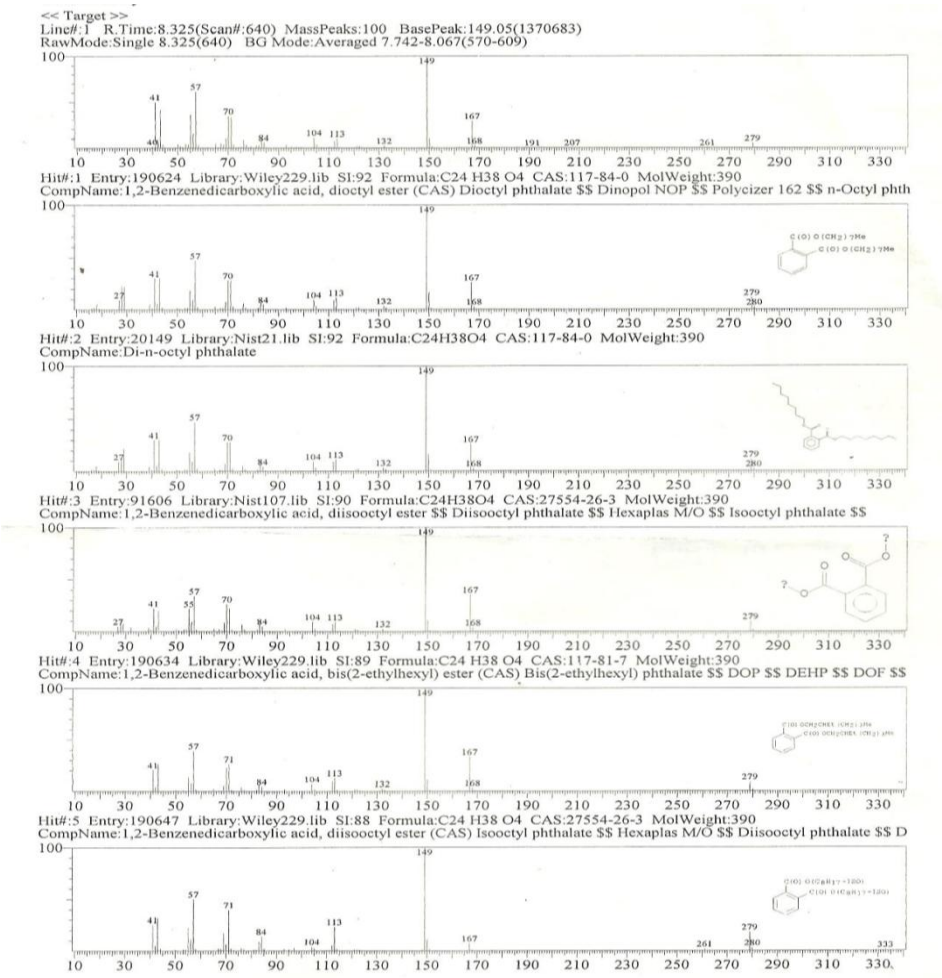


Fig 2. The spectra of major compounds (90.21%) with molecular weight 390.

S No.	RT	Name of the Compound	Molecular Formula	Molecular Weight	Biological nature
1.	8.325	1,2-Benzene dicarboxylic acid, Dioctyl ester (CAS) dioctylphthalate,dinopol NOP polycizer 162n-octylphthalate	$C_{24}H_{38}O_4$	390	Bio plasticizer
2.	8.325	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	Bio plasticizer
3.	8.325	1,2-Benzene dicarboxylic acid	$C_{24}H_{38}O_4$	390	Organic acid
4.	8.325	1,2-Benzene dicarboxylic acid, bis(2-ethyl hexyl)ester(CAS) Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	Unknown
5.	5.325	1,2-Benzene dicarboxylic acid, diisooctyl hexaplasdiisoocty pthalate	$C_{24}H_{38}O_4$	390	Unknown

6.	7.650	Decane 2,3,5,8-Tetramethyl	$C_{14}H_{30}$	198	Hydrocarbon
7.	7.650	Decane 2,3,5,8-Tetramethyl	$C_{14}H_{30}$	198	Hydrocarbon
8.	7.650	Dodecane 2,6,10-Trimethyl	$C_{15}H_{32}$	212	Hydrocarbon
9.	7.650	Nonadecane n-Nonadecane	$C_{19}H_{40}$	268	Hydrocarbon
10.	7.650	(Heptadecane 2,6,10,15-tetramethyl)2,6,10,15-Tetramethyl heptadecane 1-Indo-2-methylundecane	$C_{21}H_{44}$	296	Hydrocarbon
11.	7.217	Decane 2,3,5,8-tetramethyl	$C_{12}H_{25}I$	296	Hydrocarbon
12.	7.217	Decane 2,3,5,8-Tetramethyl	$C_{14}H_{30}$	198	Hydrocarbon
13.	7.217	Dodecane 2,6,10-trimethyl	$C_{14}H_{30}$	198	Hydrocarbon
14.	7.217	1-Octanol, 2-butyl 2-butyloctanol2-butyloctylalcohol5-	$C_{15}H_{32}$	212	Hydrocarbon
15.	7.217	(hydroxyl methyl 1) undecane	$C_{12}H_{26}O$	186	Hydrocarbon

TABLE 1. LIST OF COMPONENTS AFTER GC-MS ANALYSIS

IV. CONCLUSION

There is an increasing demand of this crop as a food product because of its slow digestibility, which is beneficial to diabetics and obese people. This is due to presence of phenolic compounds present in sorghum. The bioactive compounds have gained increased interest due to their antioxidant activity, cholesterol-lowering properties and other potential health benefits. Hence, studies on *Sorghum*, as an important source of bioactive compounds, could combat nutritional deprivation.

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