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# Estimation of Total Bioactive Compounds in Pigmented and Non Pigmented Genotypes of Sorghum (*Sorghum bicolor* (L.) Moench)

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**A B S T R A C T**

Sorghum seed contains unique bioactive compounds on par with other cereals. Bioactive compounds in plants can be defined as secondary plant metabolites. The secondary bioactive compounds in plants appear to be randomly synthesized but they are not useless junk. They can be used as anantimicrobial, antioxidant, and anti-inflammatory activity. Hence, In the present study, we have investigated to estimate the total bioactive compounds in selected genotypes by using spectrophotometer to quantitative and qualitative assay on six different genotypes including non pigmented (IS 3477, IS 33095, IS 7005) pigmented (IS 2898, IS 7155, IS 1202). The range of bioactive compounds between the pigmented and non pigmented genotypes detected high amount of total Polyphenols, Tannins, Flavonoids, Carotenoids and Anthocyaninsi.e.,40.33 ± 0.98-96.24 ± 1.97 mg GAE/100 gm, 26.61 ± 1.00-88.42 ± 1.89 mg TAE/100 gm, 2.16 ± 0.15-8.48 ± 0.16mg QUE/100 gm, 2.35 ± 0.10-21.20 ± 0.38 β-CAE/100 gm, 2.31 ± 0.22-8.49 ± 0.52C-3-G mg/100 gm respectively. Among the genotypes, pigmented genotypes showed high levels of bioactive compounds than non pigmented genotypes.



## Introduction:

According to FAO, Sorghum (*Sorghum bicolor*) ranks fifth in the world cereal grain production behind wheat, rice, maize, and barley. It constitutes the main food grain for over 750 million people who live in the semi-arid tropics of Africa, Asia, and Latin America. Sorghum is used for food, feed and fodder. Itis a single most important amongst cereal,grown in the lowland areas because of its high drought tolerance [1].

Among the cereals, sorghum is a good source of phenolic compounds with a variety of genetically dependent types and levels including phenolic acids, flavonoids, and condensed tannins. Sorghum grain (caryopsis) is composed of three main parts: seed coat (testa or pericarp), germ (embryo) and endosperm (storage tissue) (Fig. 1). In some sorghum varieties the testa is highly pigmented. The presence of pigment and the color is a genetic character controlled by the R and Y genes. The thickness of the testa layer is not uniform and is governed by the Z gene. In some varieties there is a partial testa, while in others it is not apparent or is absent [2].

Bioactive compounds in plants can be defined as secondary plant metabolites. The secondary bioactive compounds in plants appear to be randomly synthesized but they are not useless junk. Several of them are found to hold important functions in the living plants for example polyphenols. Now a days, phenolic compounds are generally regarded as desirable components of human food, because of their antioxidant activity. Therefore, they are considered to be of nutraceutical importance. Polyphenols are regarded as natural antioxidant molecules with outstanding beneficial effects, including anti-aging activity, and the prevention of cancer and diabetes. The antioxidant activity of (poly) phenols is mainly associated with their radical scavenging activity, although they may be free radical scavengers or generators, depending on their nature and concentration. This dual effect, may contribute to their influence on cell viability [3]. The most important among the bioactive compounds i.e., Anthocyanins, might be a good source of food colorants and antioxidant compounds. Anthocyanins from fruits have been shown to possess several therapeutic benefits, including vasoprotective and anti-inflammatory

properties, anti-cancer and chemoprotective properties, as well as antineoplastic properties. Therefore, the sorghum anthocyanins should be investigated for any unique health properties [4].

Phenolics are considered as a major group of bioactive compounds that contribute to the antioxidant activities of cereal grains. In cereals, Sorghum and barley are two important food grains reported to contain significant quantities of phenolic compounds [5]. Despite this, Sorghum grain and its products have not been explored extensively for their phytochemical attributes. Sorghum has unique and diverse bioactive phenolic compounds that could provide health benefits beyond standard nutrition. High levels of various phenolic compounds and antioxidant activity have been previously reported in sorghum [6, 7]. Hence, the main objective of this study was conducted on quantitative and qualitative analysis of total bioactive compounds in pigmented and non pigmented genotypes.

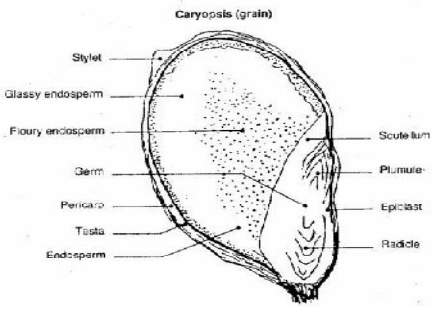


**Fig: 1.**Structure of Sorghum grain [8]

**Materials and Methods:**
**Sorghum seed sample extraction:**
**Methanol extraction:**

For the preparation of methanolic extract, the powdered material of 1kg grind with rotary miller and the powdered material extracted with aqueous methanol (80%) by soxhalation. The crude extract was evaporated to dryness in a rotary film evaporator to give a yield of 110.11gm (Sorghum). For the assessment of total bioactive compounds. The aqueous methanol extract were redissloved in methanol for qualitative and quantitative analysis of *in vitro* bioactive compounds.

**Qualitative analysis of bioactive compounds constituents:**

**Test for saponins:**

5 mL of methanolic extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins [9, 10].

**Test for anthraquinones:**

Borntrager's test: 3 mL of methanolic extract was shaken with 3 mL of benzene, filtered and 5 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones [9, 10].

**Test for Terpenoids:**

2 mL of the methanolic extract was dissolved in 2 mL of chloroform and evaporated to dryness. 2 mL of concentrated sulphuric acid was then added and heated for about 2 min. A grayish colour indicates the presence of terpenoids [9, 10].

**Test for steroids:**

A red colour produced in the lower chloroform layer when 2 mL of methanolic extract was dissolved in 2 mL of chloroform and 2 mL concentrated sulphuric acid added indicates the presence of steroids [9, 10].

**Test for alkaloids:**

3 mL of methanolic extract was stirred with 3 mL of 1% HCl on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids [9, 10].

**Test for glycosides:**

(a) Liebermann's test: 2 mL of the methanolic extract was dissolved in 2 mL of chloroform and 2 mL of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside [9, 10].

(b) Keller-Kiliani test: 2 mL of each methanolic extract was dissolved in 2 mL of glacial acetic acid containing one drop of $FeCl_3$ solution. The mixture was then poured into a test tube containing 1 mL of concentrated $H_2SO_4$. A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides [9, 10].

**Test for carbohydrates:**

(a) Molisch's test: 3 mL of the aqueous extract was added to 2 mL of Molisch's reagent and the resulting mixture shaken properly. 2 mL of concentrated $H_2SO_4$ was then poured carefully down the side of the test tube. A violet ring at the interphase indicates the presence of carbohydrate [9, 10].

(b) 3 mL of the methanolic extract was added about 1 mL of iodine solution. A purple coloration at the interphase indicates the presence of carbohydrates[9, 10].

**Quantitative analysis of bioactive compounds constituents:**

**Determination of total phenolics content:**

The content of total phenolics in extracts was determined by a modified Folin–Ciocalteu method.

Briefly, 100 µl of each extract were shaken for 1 min with 500 µl of Folin–Ciocalteu reagent and 6 mL of distilled water. After the mixture was shaken, 2 mL of 15% $Na_2CO_3$ were added and the mixture was shaken once again for 5 min. Finally, the solution was brought upto 10 mL by adding distilled water. After 2 hours, the absorbance was read on the UV/visible spectrophotometer at 750 nm (25ºC) using glass cuvettes against a blank (100 µl of distilled water instead test samples). The TPC was assessed by plotting the gallic acid calibration curve (from 1 to 1500 µg/mL) and expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract [11].

**Determination of Tannin content:**

Tannin content was determined using the vanillin-HCl method as described by Price [12]. 1 mL aliquots were mixed with 5 mL vanillin reagent, and the absorbance read at 500 nm after 20 min blank determinations were done to counteract the effect of anthocyanins and other pigments in the samples. The standard used was tannic acid; tannin content was expressed as mg tannic acid equivalents per gm (mg TE/gm) [12].

**Determination of total Carotenoids content:**

Total carotenoid contents in the extracts were determined by a spectrophotometric assay with some modifications. Approximately, 5 mL of methanolic seed extract were mixed with equal volume of distilled water and 15 mL of hexane/acetone/methanol (50/25/25, v/v) solution. The mixture was homogenized then centrifuged at 3000 rpm for 10 min. The absorbance of the top layer of hexane was measured at 450 nm using a spectrophotometer. Total carotenoid contents of the samples were calculated as mg β-carotene per 100 gm of sample [13].

**Determination of Total Flavonoid content:**

Aluminum chloride ($AlCl_3$) colorimetric method was used with some modifications to determine flavonoid content. 1mL of sample sorghum methanolic extract was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1M potassium acetate and 5.6 mL of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/mL). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent as mg/gm of extracted compound [14].

**Determination of Anthocyanin content:**

The total anthocyanin content was measured by the pH-differential method using 2 buffer systems: potassium chloride buffer, pH 1.0 and sodium acetate buffer, pH 4.5. The sample diluted with corresponding buffer and they were kept at room temperature for 15 min, the absorbance was measured at 510 and 700 nm. Total anthocyanins were calculated as mg/mL cyanidin-3-glucoside [15]

**Results:**

**Quantitative analysis:**

**Total phenolics:**

The general definition of a phenolic compound which contains a benzene ringwith one or more hydroxyl groups is known as phenolic compounds. Plant polyphenolics are well known antioxidants through electron donating properties. The total phenolic contents of sorghum grains were determined by the Folin-Ciocalteu method and an obtained results are presented in [Fig. 2] and the grains showed high amount of phenolic content in all genotypes. The polyphenolic contents in the methanolic extracts were expressed as mg of Gallic acid equivalents (GAE) per 100 gm of sample. A wide variation was observed in the total polyphenol content among the six sorghum genotypes.

The total polyphenolic contents of the samples ranged from 40.33 ± 0.98 to 96.24 ± 1.97mg GAE/100 gm (Table 1). Among all the samples, pigmented sorghumgenotypes IS2898, IS 7155, IS 1202 exhibited high amount of total polyphenols i.e., 96.24 ± 1.97mg/100 gm, 78.22 ± 1.69 mg/100gm, 69.25 ± 1.35 mg/100 gm where as non pigmented genotypes showed IS 3477, IS 33095, IS 7005 i.e., 61.22 ± 1.12 mg/100 gm, 56.12 ± 1.52 mg/100 gm, 40.33 ± 0.98 mg/100 gm. The total six genotypes of pigmented and non-pigmented sorghum, IS 2898 showed highest total phenolic content on par with other genotypes.

**Tannins:**

Tannin contents of sorghum grains were determined by the modified vanillin-HCl method and the obtained results are presented in [Table 1]. The tannins contents in the methanolic extracts were expressed as mg of tanninc acid equivalents (TAE). The statistical analysis revealed significant differences ($p < 0.05$) in tannin contents among the tested genotypes. As shown in [Fig. 3] the grains of pigmented genotypes IS 2898, IS 7155 and IS 1202 had extremely high amount of tannins 88.42 ± 1.89, 68.23 ± 1.73, 48.41 ± 0.82 mg/gm of TAE as compared with the non pigmented varieties IS 3477, IS 33095, IS 7005 in low amount of tannins 33.90 ± 0.23, 29.30 ± 1.71, 26.61 ± 1.00 mg/gm TAE respectively.

**Flavonoids:**

Total flavonoids contents in the sorghum methanolic extract grains was expressed as mg of Quercetin/100 mg. Among the genotypes, the pigmented and non pigmented genotypes of sorghum range between 2.16 ± 0.15-8.48 ± 0.16 mg quercetin/100gm were shown in [Table 1]. The flavonoid content was highest in the pigmented genotype IS 2898 (8.48 ± 0.16 mg/100 gm) followed by IS 1202 (7.55 ± 0.33 mg/100 gm), IS 7155 (4.56 ± 0.10 mg/100

gm) IS 3477 (3.48 ± 0.23 mg/100 gm), IS 33095 (2.80 ± 0.18 mg/100 gm), IS 7005 (2.16 ± 0.15 mg/100 gm) were shown in [Fig. 4].

**Carotenoids:**

Carotenoids may act as singlet oxygen quencher and can transfer one electron to the radicals, giving rise to a stable carotenoid radical cation regenerating the original molecule. Total carotenoid contents in grains, expressed as mg of β-carotene equivalents per 100 gm of sample were significantly lower in all the tested samples 2.35 ± 0.10-21.20 ± 0.38 mg β-carotene/100 gm than the total phenolics, tannin and flavonoid contents [Table 1]. In our study, among the six genotypes, pigmented genotype IS 2898 had higher content and non pigmented genotype IS 7005 had lower content of carotenoids were identified [Fig. 5].

**Anthocyanins:**

The amount of total Anthocyanins content ranged widely in each sorghum grains were shown in [Table 1] and [Fig.6]. The calculated values were 3.32 ± 0.24, 2.89 ± 0.19, 2.31 ± 0.22, 8.49 ± 0.52, 6.89 ± 0.18, 5.18 ± 0.41 as mg Cyaniding-3-glucoside/100 gm for genotypes IS 3477, IS 33095, IS 7005, IS 2898, IS 7155 and IS 1202 respectively. With the exception of variety IS 7005, the other varieties showed significant differences ($p < 0.05$) in anthocyanin contents. The total six genotypes, grains of pigmented varieties IS 2898, IS 7155 and IS 1202 were rich in anthocyanin contents. They might be a good source of food colorants and antioxidant compounds. Among the pigmented genotypes, IS 2898 which showed high amount of anthocyanins content.

**Qualitative analysis:**

Qualitative tests were carried out on the grains of pigmented and non pigmented genotypes using standard procedures to estimate the constituents as described by according to methods [9, 10]. The bioactive components screening results are illustrated in [Table. 2]. The number of positive signs indicated the intensity of the reactions that reflects the quantity present. The chemical tests indicated the presence of alkaloids, triterpins, steroids, carbohydrates, glycosides and anthraqunones in all samples. The test for anthraquinones were present in only pigmented genotypes i.e., IS 2898, IS 7155, IS 1202. The Saponins content was absent in all the tested genotypes. However, further studies are needed in order to isolate, identify, characterize and elucidate the structure of these compounds.

However, this study suggests that sorghum pigmented genotypes could provide a unique opportunity to produce special food products with a natural, attractive dark color, high levels of polyphenols, tannins, flavonoids, carotenoids and anthocyanins present in pigmented genotypes than non pigmented genotypes. Hence these genotypes could be novel sources of bioactive components.

**Discussion:**

In the present study, we found that total phenolic content of methanolic extracts of sorghum (Folin–Ciocalteu method) was similar to that reported by the Prussian blue method [16]. Sorghum from red and purple plants had higher levels of total phenols [17, 18, 19] Where as in our study, the polyphenolic content range between 40.33-96.24 mg/gm. We also observed pigmentd seed coat testa genotypes showed high levels of bioactive compounds. The levels of total phenols in our study of the sorghum genotypes was 3 times higher than in fruits and berries [17]. The similar study was conducted on high levels of polyflavanols (970 mg CE/gm) in sorghum [20], while that both brown sorghum grains and brans had high proanthocyanidin contents (21-58 mg/gm) compared to blueberry (20 mg/gm) [20]. The effect of pigmented testa and pericarp color on total phenol content was consistent. Other attributes that affect total phenol distribution among sorghum genotypes include pericarp thickness and plant colors a positive correlation between pericarp thickness and total phenol.

Generally, the extraction of an antioxidants in grains are very difficult due to different solubility of active compounds [21]. Among organic solvents including n-hexane, diethyl ether, ethyl acetate, acetone water methanol. The use of methanol as solvent extractor showed high antioxidant activity was observed by previously reported by different workers [22, 23]. Therefore, we have chosen methanol as an extraction solvent. In our investigation, among all the samples, pigmented sorghum genotypes (IS 2898, IS 7155 and IS 1202) displayed detectable amounts of bioactive compounds like total polyphenols, tannins, anthocyanins, flavonoids and carotenoids. It indicates methanol appears to be the best solvent for extracting compounds such as phenolics, flavonoids and other polar material in cereals [24, 25].

Tannin contents of sorghum grains were determined by the modified vanillin-HCl method. In the present study, the tannin content in all genotypes was tested among these pigmented genotypes were showed high level of tannins the range between 26.61-88.42 mg/gm TAE. As expected from previous studies in all the samples contained considerable amounts of tannin contents. In sorghum, pericarp color, endosperm color, and the presence of a pigmented testa are factors affecting the color and acceptability of food products [26, 27]. Recent studies strongly indicated that tannins are of benefit to human health. Tannins bind to proteins and make them indigestible. However, *in vitro* data indicate that the microflora in the colon degrade polymeric tannins into low molecular weight phenolic acids which could be absorbed through the colon. Tannins are nontoxic and may slow digestibility in humans, which is an advantage for type II diabetics [28]. In the present investigation, Grains of pigmented

genotypes IS 2898, IS 7155 and IS 1202 were rich in anthocyanin contents the range between 2.31-8.49 mg/gm. Our study results correlate with the previous reports of anthocyanin levels of 4.7 mg/gm [29, 30, 31].

The using of sorghum grains, reported higher levels of flavan-4-ols (0.20-0.42%, w/w, cyanidin, dry weight basis) in red-plant sorghums with a red pericarp and pigmented glumes than the other varieties studied [32, 33]. Higher values observed in red and black pericarp sorghum may be attributed to the high levels of flavan-4-ols and 3-deoxyanthocyanins contributing to the antioxidant activity [34, 35]. Carotenoids may act as singlet oxygen quencher and can transfer one electron to the radicals, giving rise to a stable carotenoid radical cation regenerating the original molecule [36]. In our study, similarly showed high level of flavonoid and carotenoid contents in pigmented genotypes as compare to non pigmented genotypes.

**Table: 1.** Quantitative analysis of total Phenolics, Tannins, Flavonoids, Carotenoids and Anthocyanins

| Sorghum genotypes | Total phenolics mg/gm | Tannins mg/gm | Flavonoids mg/gm | Carotenoids mg/gm | Anthocyanins mg/gm |
|---|---|---|---|---|---|
| IS 3477 | 61.22±1.12 | 33.90 ± 0.23 | 3.48 ± 0.23 | 10.22 ± 0.33 | 3.32 ± 0.24 |
| IS 33095 | 56.12±1.52 | 29.30 ± 1.71 | 2.80 ± 0.18 | 5.66 ± 0.54 | 2. 89 ± 0.19 |
| IS 7005 | 40.33±0.98 | 26.61 ± 1.00 | 2.16 ± 0.15 | 2.35 ± 0.10 | 2. 31 ± 0.22 |
| IS 2898 | 96.24±1.97 | 88.42 ±1.89 | 8.48 ± 0.16 | 21.20 ± 0.38 | 8.49 ± 0.52 |
| IS 7155 | 78.22±1.69 | 68.23 ± 1.73 | 4.56 ± 0.10 | 18.55 ± 0.66 | 6.89 ± 0.18 |
| IS 1202 | 69.25±1.35 | 48.41 ± 0.82 | 7.55 ± 0.33 | 15.25 ± 0.52 | 5.18 ± 0.41 |

**Table: 2.** Qualitative analysis of total bioactive compounds

| Sorghum genotype | Alkaloids | Saponins | Triterpins | Steroids | Carbohydrates | Glycosides | Anthraquinones |
|---|---|---|---|---|---|---|---|
| IS 3477 | + | - | + | + | + | + | - |
| IS 33095 | + | - | + | + | + | + | - |
| IS 7005 | + | - | + | + | + | + | - |
| IS 2898 | + | - | + | + | + | + | + |
| IS 7155 | + | - | + | + | + | + | + |
| IS 1202 | + | - | + | + | + | + | + |

**Conclusion:**

Total bioactive compounds of the whole sorghum seed has not been done so far, the present study provides a significant effect of the use of these sorghum seed extracts in traditional health care system. The present investigation also suggests that seed of sorghum may be utilized as effective and safe natural source for antioxidant and antimicrobial agents. Pigmented sorghum genotypes showed high amount of bioactive compounds which can be applied in both healthy medicine and food industry.

**Acknowledgements:**

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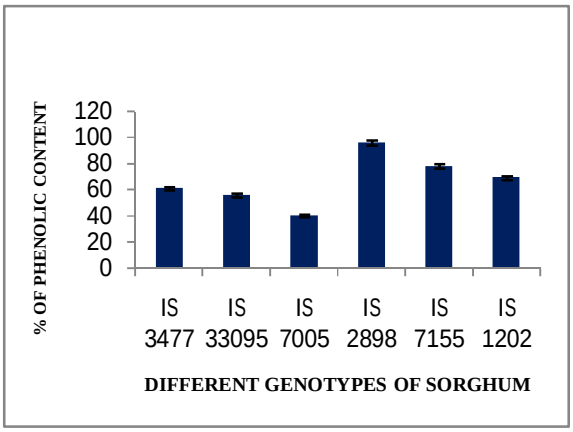


**Fig: 2. Total phenolics content**

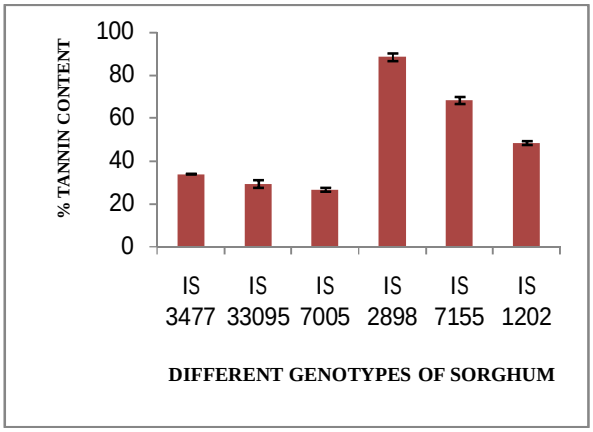


**Fig: 3. Total Tannin content**

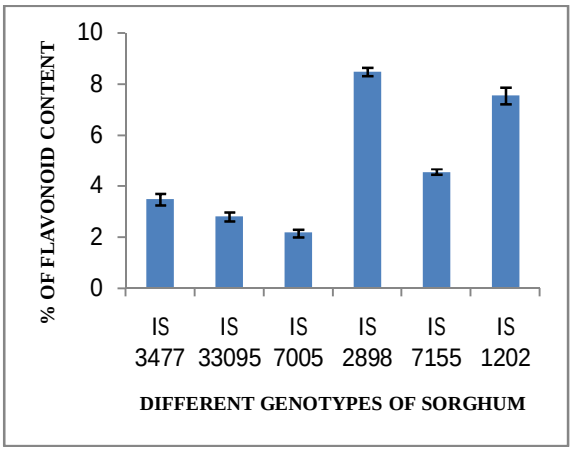


**Fig: 4. Total Flavonoids content**

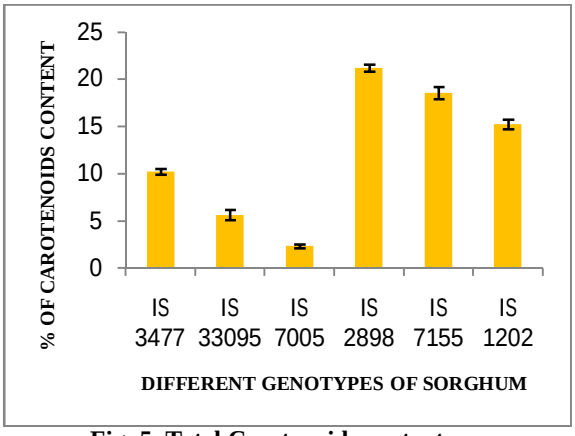


**Fig: 5. Total Carotenoids content**

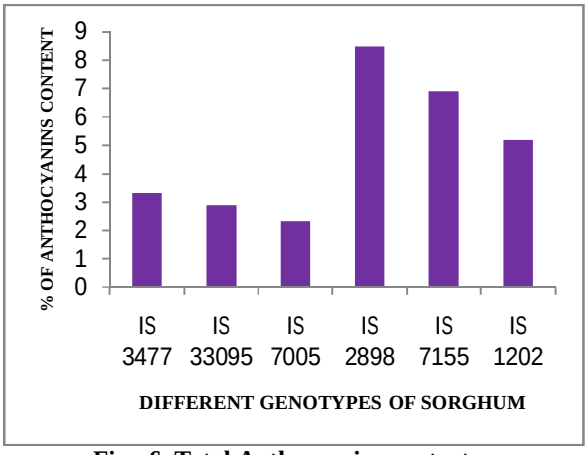


**Fig: 6. Total Anthocyanins content**

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