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RESEARCH ARTICLE

Comparative study of proximate composition and total antioxidant activity in leaves and seeds of *Oryza sativa* and *Myriostachya wightiana*.

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Abstract

The world largest and most prominent cereal crop with agricultural and economic importance as a staple food for more than 50% population worldwide is Rice. *Myriostachya wightiana*, belongs to the family Poaceae, is a perennial grass grows in inter-tidal mangrove swamps. The current investigation was undertaken to evaluate and compare the Chlorophyll, Proximate composition and total Antioxidant potential of leaves and seeds of *Oryza sativa* and *Myriostachya wightiana*. Specific extraction and estimation methods were used to quantify the proximate composition. The results analysed with Student's f-test for paired data and 'p' value less than 0.05 was considered as significant difference. Maximum chlorophyll content was observed in leaves of *Oryza sativa* (23.87 mg/gm). Superior amounts of starch, reducing and non reducing were found in seeds of Oryza sativa. While the better protein content was found in the leaves and seeds of *Myriostachya wightiana*. When compared with *Oryza sativa*, low quantity of lipid was found in seeds of *Myriostachya*. Elevated free proline was found in the leaves and seeds of *Oryza* and *Myriostachya*. The total antioxidant potential was found high in leaves and seeds of *Myriostachya*. The nutritive value of rice and *Myriostachya* seeds were quantified as 313.6 and 282.5 K.Cal/100gm correspondingly.

Introduction:-

In worldwide more than 50% of the population directly depends on the rice for their food. More than 90% of the rice cultivated by the farmers in Asia. Two countries, India and China, grow more than half of the total crop (IRRI, 2011). In addition to its food value and economic importance, rice has relatively small genome size and complete genome sequence Sasaki (2005), is considered as an experimental monocot system for various biotechnological, metabolic, genetic engineering, functional genomics and development studies worldwide. Bajaj and Mohanty (2005).

The rice grain consists embryo, endosperm, bran, outer grain layers and inedible fibrous hull. In many countries the majority of the people consumed rice in its polished form. Polishing refers to the mechanical processing of the grain by first removing the hull from the grain to get milled rice. Polishing is done to prevent rancidity of the rice oil in the outer layers of the grain and also for the consumers' preferences. Montilla (2006). The predominant form of rice found in today's market is, milled or polished rice. Polishing leads to the considerable loss of several nutritional valuable components, which are mostly concentrated in the germ and outer layers than in the starchy endosperm.

Myriostachya wightiana is one of the important salt marshes in mangrove areas belonging to the family Poaceae. It is a perennial grass grows along the muddy creeks and channels in intertidal mangrove swamps of India, Bangladesh and extending into Myanmar, Malaysia and Vietnam. In India it is mainly distributed in east coast of the Bay of Bengal, South India. Siddiqui (2001). It favours more saline water than fresh water for its growth and development and provides habitat for many fishes and other organisms. Commonly used as a fodder grass and thatching material

and ecologically very important for phytoremediation and checks soil erosion effects. Sahu (2015). The mangroves are excellent feed for cattle. The buffaloes, goats and cow are left among mangroves during the summer months and they graze Avicennia leaves and grasses (Porteresia coarctata, *Myriostachya wightiana*, Aleuropus lagopoides). Banerjee et al (1998). Naturally adapted salt tolerant plants provide an excellent material for investigating the adaptive mechanism they use to encounter salinity. *Myriostachya wightiana* showed a large range of anatomical adaptive features. Rashid and Ahmed (2011).

In this study, the biochemical parameters like chlorophyll, carbohydrates, total lipid, total protein, free proline and total antioxidant activities both in *Myriostachya wightiana* and *Oryza sativa* were investigated.

Materials & Methods:-

Chemicals:-

Chemicals and reagents (analytical grade) used for biochemical, antioxidant activity were purchased from Sigma Aldrich. The experiments were performed at room temperature otherwise stated.

Sample collection:-

Rice sample (*Oryza sativa* L.) was collected by the AP Rice Research Institute, Marteru, West Godavari Dist, Andhra Pradesh, India. *Myriostachya wightiana* was collected from Bhavanapadu creek, Tekkali, Andhra Pradesh, India..

Estimation of chlorophyll in leaves:-

The chlorophyll content in the leaves was estimated by Arnon (1949) method. Fully expanded leaves were washed thoroughly with distilled water. The chlorophyll was extracted with 80% acetone. The absorption of the extracts measured spectrophotometrically at 663 nm (D_{663}) and 645 nm (D_{645}). The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b) and the total chlorophyll (Chl t) were calculated using the equation as below

Chl a (mg/g) = [(12.7 X A_{663}) - (2.6 X A_{645})] X ml acetone / mg leaf tissue

Chl b (mg/g) = [(22.9 X A_{645}) - (4.68 X A_{663})] X ml acetone / mg leaf tissue

Chl t (mg/g) = [(20.2 X A_{645}) - (8.02 X A_{663})] X ml acetone / mg leaf tissue

Estimation of Reducing sugars:-

Reducing sugars were estimated by the method of Miller (1972). 0.1 gm fresh leaves and seeds of *Myriostachya wightiana* and *Oryza sativa* were homogenised separately in 80% hot ethanol and centrifuged at 5000g for 15 min at room temperature. The supernatant was evaporated by keeping in water bath at 80^0C and sugars were dissolved by adding 10 ml distilled water. 1 ml of solution from both the samples were taken in separate tubes and 3 ml of DNSA reagent was added, then boiled for 5 min in a water bath. After boiling 1 ml of Rochelle salt solution was added. The tubes were cooled to room temperature and measured the intensity of dark red colour at 530 nm and calculated the concentration of reducing sugars from glucose standard graph.

Estimation of Nonreducing sugars:-

Hedge and Hofreiter (1962) method was used to estimate the Nonreducing sugars. leaves and seeds of *Myriostachya wightiana* and *Oryza sativa* (0.25 gm) were hydrolysed separately by keeping in water bath for 3 hrs, with 2.5 N Hcl (5 ml) and was neutralised with Na_2CO_3 after cooled it to room temperature. 0.1 ml extracts were taken in separate test tubes and makeup to volume 1 ml with distilled water and 4 ml of anthrone was added. The tubes were kept for boiling for 5 min. colour intensity was measured at 630 nm. Non reducing sugars concentration was calculated from glucose standard graph.

Extraction and estimation of starch:-

Thayumanavan and Sadasivam (1984) method was used to estimate the starch by using anthrone method. 250 mg fresh leaves and seeds of *Myriostachya wightiana* and *Oryza sativa* homogenised separately in 80% hot ethanol to remove sugars. Residue was retained after centrifugation at 5000 X g for 15 min. The starch was extracted by 52% perchloric acid at 0^o C for 20 min. 0.1 ml of extract was taken in separate test tubes and the final volume was adjusted to 1 ml with distilled water and 4 ml of anthrone was added. The tubes were kept for boiling for 5 min. The colour intensity was measured at 630 nm. Starch concentration was calculated from the glucose standard graph.

Extraction and estimation of total protein:-
Ferreira et al (2002) method was used to extract the total protein by using poly vinyl pyrrolidone (PVP). 0.5 gm leaves and seeds of *Myriostachya wightiana* and *Oryza sativa* were homogenised separately in 50 mM sodium phosphate buffer containing 10% insoluble PVP and incubated at 40^0 C for overnight. Then the homogenates were centrifuged at 14000 rpm for 20 min at 4^0 C. The supernatant was stored at -20^0C and used for protein quantification. The total protein concentration was estimated by using Lowry et al (1951) method.

Extraction and estimation of total lipid:-
Bligh and Dyer (1959) method was used for the extraction of total lipids from the leaves and seeds of *Myriostachya wightiana* and Oryza sativa. 1 gm leaves and seeds were homogenised separately with 3.75 ml methanol: chloroform (2:1V/V) and 1 ml of 1 mM EDTA in 0.15 M acetic acid was added. Homogenate was transferred to new glass tube and the homogenizer rinsed with 1.25 ml of chloroform and transferred to the tube, finally 1.25 ml of 0.88% Kcl was added and centrifuged at 3000 rpm for 2 min. The lower phase was transferred to new tube. The total lipid concentration was estimated by the method of Kinght et al (1972) by using phosphovanillin.

Total Antioxidant Activity:-
The Total Antioxidant Potential of leaves and seeds were estimated separately by the method of Prieto et al (1999). The assay was based on the reduction of Mo (VI) to Mo (V) and formation of a green complex at acidic pH. The tubes containing methanolic extracts and reagent solution (0.6 M H2SO4, 28 mM sodium phosphate and 4mM ammonium molybdate) were incubated at 95^0C for 90 min, again cooled to room temperature (25^0C). The absorbance measured at 695 nm. The antioxidant activity was expressed as Ascorbic Acid Equivalents (AAE).

Estimation of free proline:-
Free proline was estimated by Bates etal (1973) method. 0.5 gm fresh leaf and seed samples were homogenized in 5 ml of 3% sulphosalicylic acid. 2 ml of extracts were taken separately in a test tubes and to it 2 ml glacial acetic acid and 2 ml ninhydrin were added. The reaction mixture was boiled in a water bath at 100^0C for 30 min. After cooling the reaction mixture, 4 ml of toluene was added. After thorough mixing, the chromophore containing toluene was separated and of red colour developed was measured spectrophotometrically at 520 nm against toluene as blank. The proline concentration was determined using a calibration curve of L-proline. Results expressed as mg of proline per gm fresh weight of tissue.

Estimation Nutritive Energy:-
After the estimation of protein, fat and carbohydrate, the nutritive value was calculated as per the following formula. Nutritive value (K.Cal per 100 g) = 4 (Protein %) + 9 (Fat %) + 4 (Carbohydrate %).

Statistical analysis:-
The results of in vitro studies were given as Mean ± Standard Deviation (SD) obtained from three independent experiments, and analyzed with Student's f-test for paired data and a 'p' value less than 0.05 was considered as significant difference in the analysis.

Results and Discussion:-

Estimation of Chlorophyll:-
Chlorophyll attentiveness showed a large variation between *Oryza sativa* and *Myriostachya wightiana*. Chlorophyll 'a' concentration of *Oryza sativa*, *Myriostachya wightiana* were 17.32 mg/gm (72.5%) and 7.86 mg/gm (57.64%) respectively. Chlorophyll 'b' is 6.53 mg/gm (27.3%) and 5.8 mg/gm (42.52%) correspondingly. The results were displayed in Fig 1. Similar results were reported by Comar (1942), that chlorophyll a lies between 67% and 78% of the normal green tissues of higher land plants. Gross (1991) stated that in higher plant, chlorophyll 'a' is the major pigment and chlorophyll 'b' is an accessory pigment and the a/b ratio is generally 3:1. Lin (2002) studies revealed that the chlorophyll a/chlorophyll b ratio were generally around 3:2. Shibghatallh (2013) reported that the concentration of chlorophyll 'a' is more comparable with chlorophyll 'b'. According to Farabee (2013) chlorophyll 'a' have significant responsibility than chlorophyll 'b' in the photosynthetic process. Chlorophyll 'a' absorbs energy in the range of violet-blue to reddish orange wave lengths, and shows little absorbance in the of intermediate wavelengths (green-yellow-orange). Whereas accessory pigments including chlorophyll 'b' absorbs energy that chlorophyll 'a' does not absorb. Molazen (2010) illustrated that chlorophyll content decreased under saline conditions. Drought stress decreases the light harvesting capacity by decreasing the total chlorophyll content. Since the production of reactive oxygen species mainly produced by excess energy absorption in the photosynthetic

apparatus, this could be lowered by degrading the absorbing pigments. Herbinger etal (2002). The results are in agreement with Nyachiro etal (2001), who described a significant decrease of chlorophyll 'a' and 'b' because of water deficit in six Triticum aestivum cultivars.

Estimation of sugars:-

Sugars confirmed a large variation between *Oryza sativa* and *Myriostachya wightiana* seeds and leaves. The concentration of reducing sugars varies in *Oryza sativa* and *Myriostachya wightiana* highest content of reducing sugars observed in seeds of *Oryza sativa* with 77±0.3 mg/gm (7.7%). The results were put on show in Fig 2. The results are in agreement with the findings of Rajakumar (2013). Maximum non reducing sugars observed in seeds of *Oryza sativa* 635±30 mg/gm (63.5%). The results were put on show in Fig 3. Seeds of *Zizania aquatica* had 540±7.5 mg/gm (54%). *Zizania aquatica* (wild rice) is an aquatic cereal, distantly related to normal cultivated rice with significant medicinal values, is an annual grass growing in shallow lakes and sluggish streams. *Myriostachya wightiana*, which resembles *Zizania aquatica* is an inhabitant of North America. The proximate composition of *Myriostachya wightiana* is unexplored till now. We took the initiative of establishing the proximate composition of *Myriostachya wightiana*. During our research, we found that the proximate composition of *Myriostachya wightiana* and *Zizania aquatica* resembles in certain aspects this is due to their habitats i.e., they both grow under saline environment. The leaves of cultivated rice show very less nonreducing sugars than leaves of *Myriostachya retina*. The highest amount of starch found in seeds of *Oryza sativa* 655±14 mg/gm (65.5%). The results were put on show in Fig 4. *Myriostachya wightiana* seeds have 565±14 mg/gm or 56.5% of starch. *Oryza sativa* leaves had very less starch than *Myriostachya wightiana* leaves. Our findings will agree with the reports of previous works. Srinivasa Rao (1971) reported that the starch content of the rice grains ranged between 70 and 76.5%. Cornelia Kennedy (1924) stated that the wild rice (*Zizania aquatica*) grains have total starch in the range of 62.03 to 65.26%, whereas cultivated rice have 69.50 to 79.00%. Findings of Robbins (1971) revealed that the starch content of wild rice varies from 60 to 65%. Anderson (1975) reported that total carbohydrates in grains of wild rice about 75% this includes starch, sugars and all other carbohydrate substances.

Estimation of proteins:-

Among seeds and leaves of *Oryza sativa* and *Myriostachya wightiana*, high amount of protein present in leaves of the *Myriostachya wightiana* by 110±4 mg/gm (11%). The results were put on show in Fig 5. *Myriostachya wightiana* seeds have a greater amount of protein than seeds of cultivated rice. Our findings agree with the results of earlier works. Patil (2014) reported that a wide variation was found in protein content of all the seeds of 58 rice varieties, varying from 6.09 to 11.2%. Banerjee (2011) reported that the protein content of milled grains among the 258 rice lines ranged from 4.91 to 12.08% with the mean of 6.63%. Findings of Cornelia Kennedy (1924) stated that the wild rice grains have protein in the range of 14.4 to 13.36%, it shows similarity with *Myriostachya wightiana* and cultivated rice grains with 5.04 to 8.0%. Lourdes (1970) reported that the protein content of the dehulled matures rice (*Oryza sativa*) grains ranged from 8.49 to 11.7%. Vidhyasekeran (1984) observed the protein content of healthy rice seeds as 8%. Chellappam Gopalakrishnan (2009) found that the healthy seeds of *Oryza sativa* have 96.57 mg/gm of protein content. Anderson (1975) reported that the wild rice seeds contain 12.4 to 15.0% protein, whereas Brown rice seeds have 7.5% and polished white rice has 6.7% only. Riza et al (2003) observed 6.3 to 9.1% grain protein levels in 438 rice cultivars.

Estimation of lipids:-

Rice seeds have the maximum amount of lipids that is 1.7 mg/gm (0.17%) than *Myriostachya wightiana* with 0.52 mg/gm (0.052%). Among the leaves *Myriostachya wightiana* show elevated levels with 3.435 mg/gm (0.34%). The results were shown in Fig 6. Our results agree with the previous reports. Anderson (1975) reported that the wild rice seeds have very low content (0.5 - 0.8) of fat than Brown rice. linoleic and linolenic acids occupy 65% of the wild rice total fatty acids which are extracted with hexane. Linolenic acid is one of the essential fatty acid for humans, the presence of high linolenic acid levels in wild rice surely improves the nutritional quality of the food. Lindsay, Smith (1975) observed that wild rice lipid is unique when compared to white rice, wheat and oats, due to its high levels of linolenic acid (30%). Anderson (1975) and that Megat Rusydi (2011) reported that the non germinating grains of white rice, black rice, red rice, and brown rice have 1.42 ± 0.04, 1.29 ± 0.02, 1.5 ± 0.09, 1.89 ± 0.04, 0.14 ± 0.01 lipid content in that order. A study of Frei and Becker (2004) on Philippine rice land races demonstrated that the average lipid content was significantly higher than that of the HYVs collected from the same area. While for all of the HYVs (Brown rice) lipid content ranged between 2.0 and 2.1%.

Total antioxidant capacity:-

Myriostachya wightiana leaves and seeds had potential antioxidant capacity. *Myriostachya wightiana* exhibit nearly double than that of *Oryza sativa*. The results were put on show in Fig 7. Our results colinear with previous works. The Reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity. Ho, Huang, (2012). Deepanjan Banerjee (2008) accounted that the antioxidant activities of 23 extracts from leaves of six mangroves and four mangrove associates, put on view in the range of 5.71 to 0.60 mg/gm of Ascorbic acid equivalents/dry tissue. Our results coordinate with the above results. *Zizania* is a mangrove associate grass shows 1.37 ± 0.022 mg/gm of AAE. Alak Kanti Dutta (2012) evaluated antioxidant activities of two Srilankan rice varieties aman and bore, which had total antioxidant capacities in the range of 297.04 ± 19.53 to 701.16 ± 1.44 µM AAE/100gm.

Total free proline:-

Rice leaves have a high amount of free proline with 1.68 mg/gm than *Myriostachya wightiana* by 1.08 mg/gm. Among the seeds *Myriostachya wightiana* showed high free proline with 0.236 mg/gm. The results were displayed in Fig 8. Our results show similarity with previous works. Proline is known as osmoprotectant and plays an important role in osmotic balancing, protection of sub cellular structures, enzymes and in increasing cellular osmolarity that provide the turgor necessary for cell expansion under stress conditions. Matysik et al (2002), Sai ram and Tyagi (2004), Studies of Juliano and Bechtel (1985) revealed that proline content varies in different rice varieties as 3.9 to 6.3% of total protein. Upadhyaya (2007) accounted proline range from 3 – 4 mg/gm in leaves of *Oryza sativa* at 0 mm concentration of H_2O_2. Priya Gurumoorthy (2014) reported that the amount of proline in the standard sample was found to be 1200.33± 26.53 µg/0.1 gm of the rice leaf tissue. Vijayarengan (2012) reported that the minimum proline content of rice leaves was recorded at 0.176 mg/gm fresh weight in control. Anderson (1975) reported that the Average proline content in different wild rice seeds as 3.9% of total protein. According to OECD, Environment Directorate the proline concentration ranges from 3.9 – 6.3% of protein in different varieties of rice seeds. It may be argued that proline accumulation helps to conserve nitrogenous compounds and protects the plant against heavy metal stress. These results also support the view that proline acts as a membrane stabilizing agent under stress conditions. Poschenrieder and Barcelo (2004).

Nutritive Energy:-

Among seeds and leaves of *Oryza sativa* and *Myriostachya wightiana*, Rice seeds have a higher calorific value that is 313.6 K.Cal/100 gm. The *Myriostachya wightiana* seed has 282.5 K.Cal/100 gm. Lowest nutritive energy 75.51 K.Cal/100 gm found in the leaves of *Oryza sativa*. The results were put on show in Fig 9. Our findings obey the earlier reports. According to FAO, (1993) Calorific value for Rough rice, Brown rice and milled rice were reported as 378, 363 – 385, 349 – 373 K.Cal respectively. Anderson (1975) reported the calorific value of wild rice as 353 K.Cal/100 gm. Creal straws like wheat bhoosa and paddy contain 3% digestible protein, 40% TDN and can meet the maintenance requirements of adult cattle and buffaloes. Since these by products contain 40-100% more DCP, they can as well meet the production requirements of the animals to a certain extent.

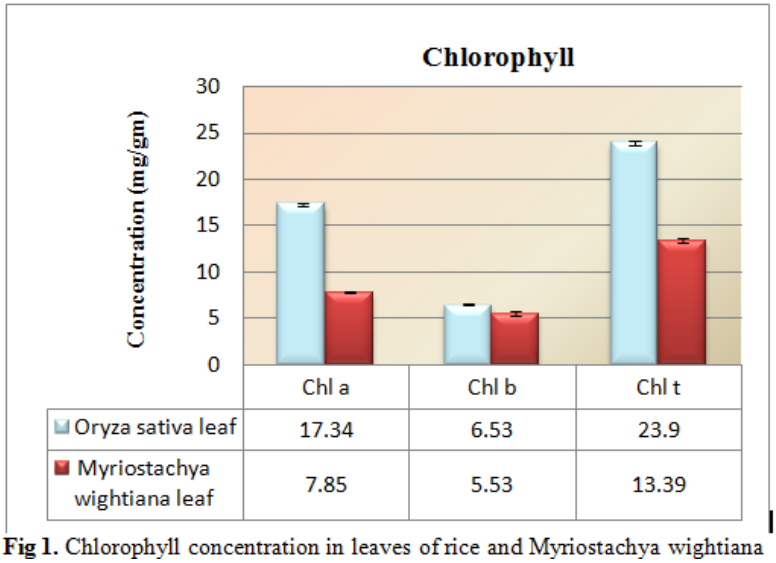

Fig 1. Chlorophyll concentration in leaves of rice and Myriostachya wightiana

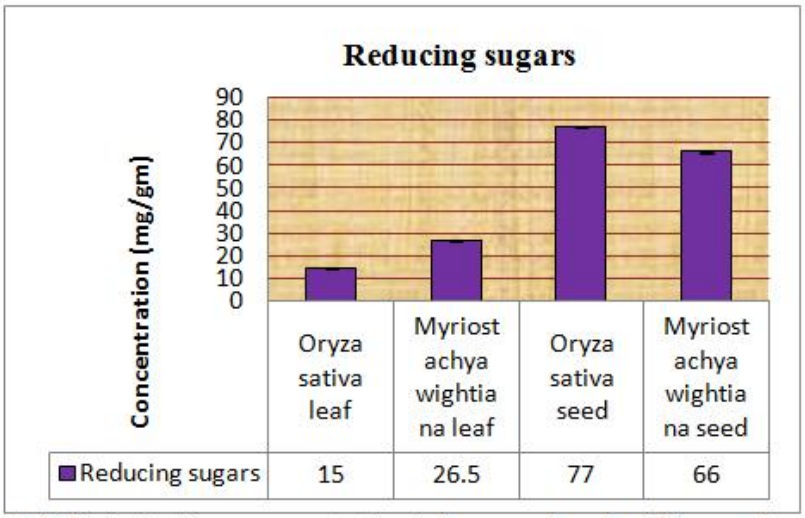

Fig 2. Reducing Sugar concentration in leaves and seeds of Oryza sativa and Myriostachya wightiana.

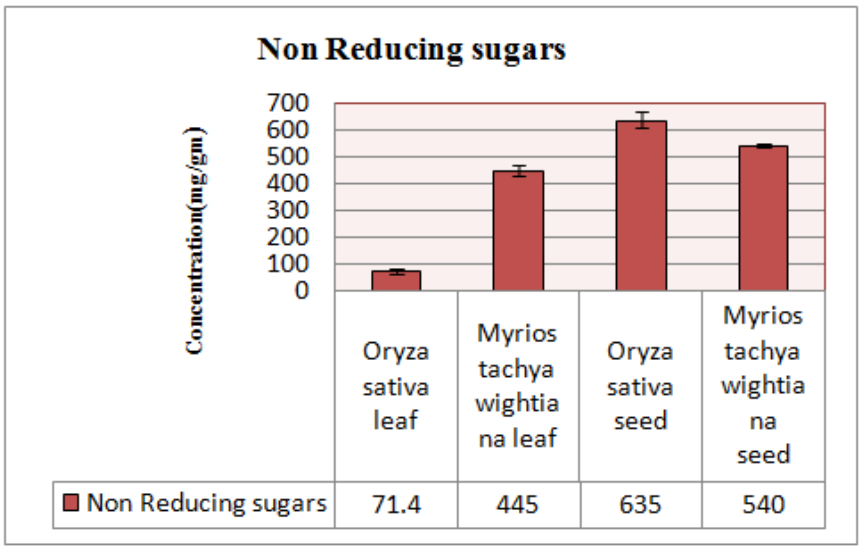

Fig 3. Non Reducing Sugar concentration in leaves and seeds of Oryza sativa and Myriostachya wightiana.

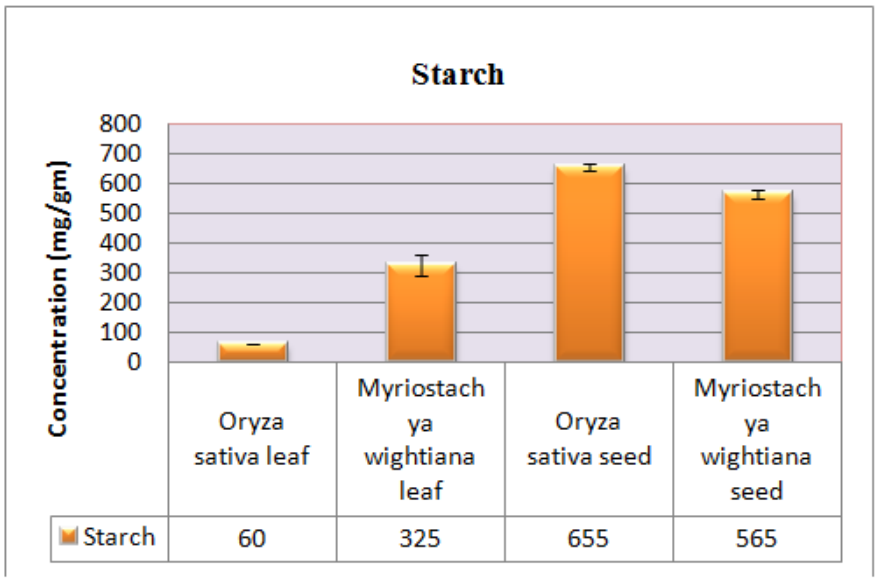

Fig 4. Starch concentration in leaves and seeds of Oryza sativa and Myriostachya wightiana.

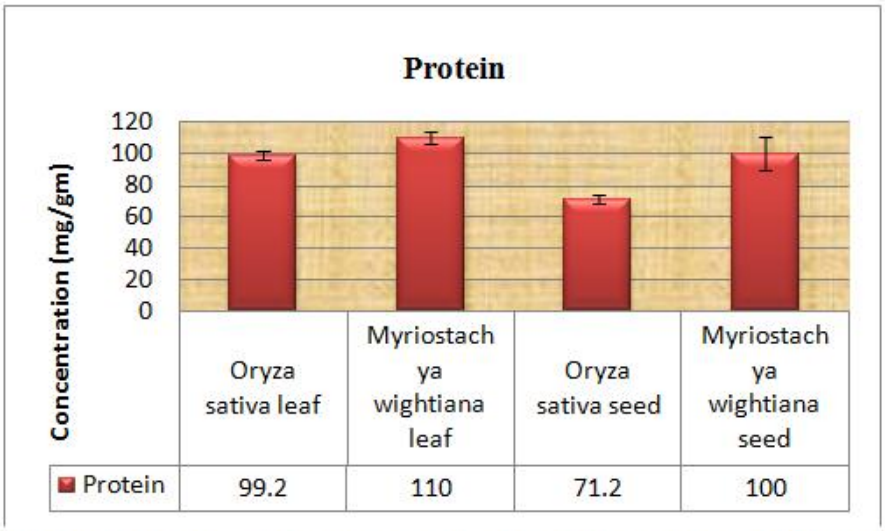

Fig 5. Protein concentration in leaves and seeds of Oryza sativa and Myriostachya wightiana.

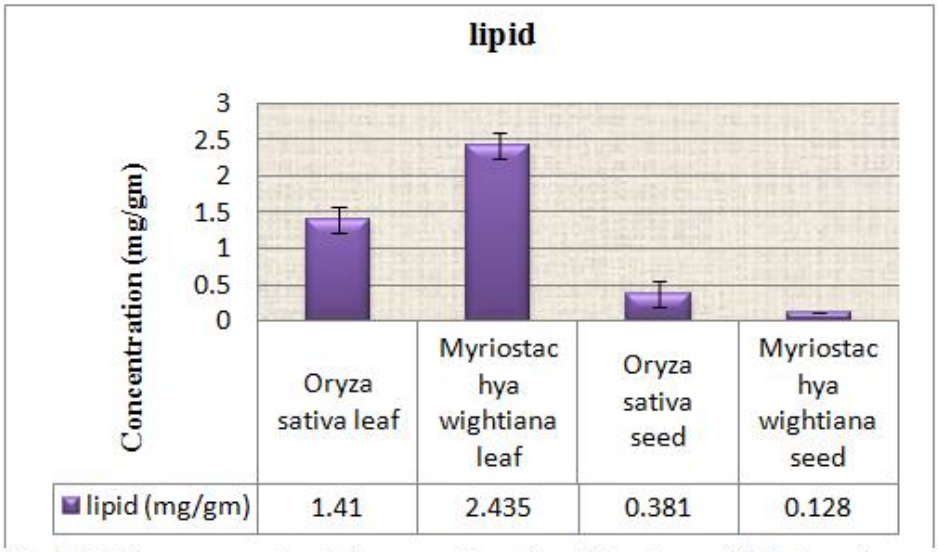

Fig 6. Lipid concentration in leaves and seeds of O.sativa and Myriostachya wightiana.

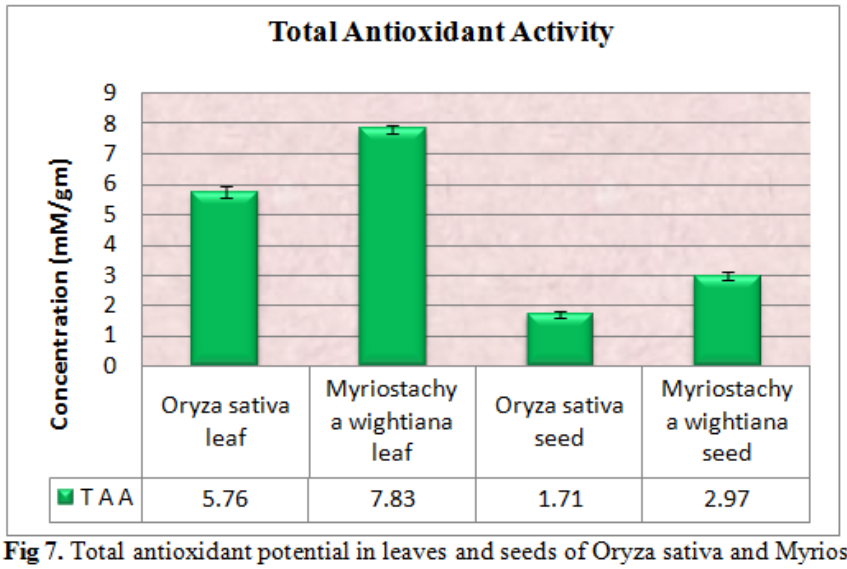

Fig 7. Total antioxidant potential in leaves and seeds of Oryza sativa and Myriostachya wightiana

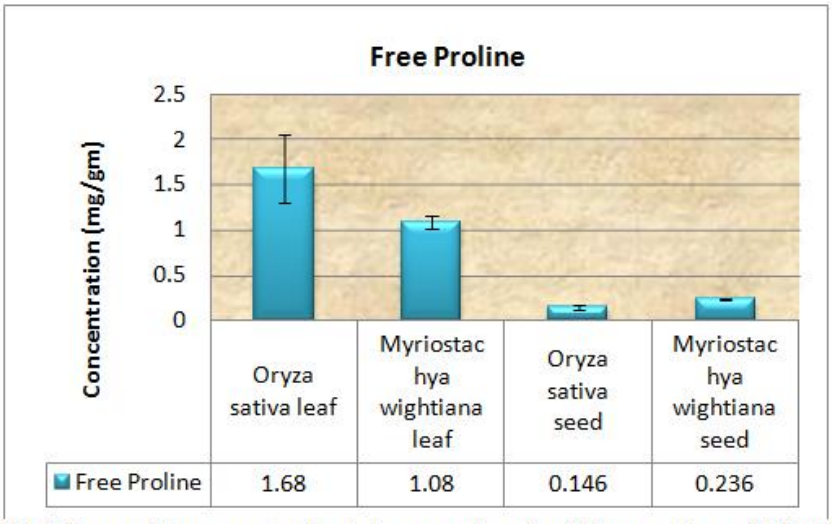

Fig 8. Free proline concentration in leaves and seeds of Oryza sativa and Myriostachya wightiana

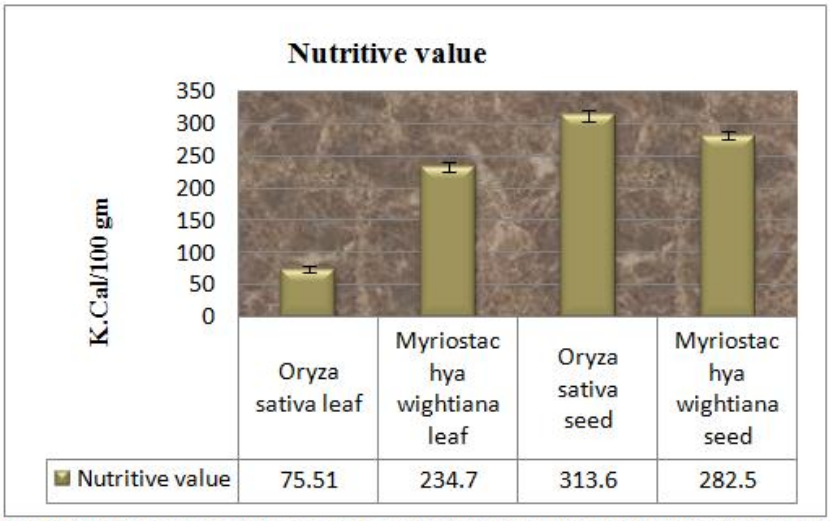

Fig 9. Nutritive value of leaves and seeds of Oryza sativa and Myriostachya wightiana.

Table: 1. Proximate composition and Total Antioxidant Activity of O.sativa and M.wightiana.

S.No	Parameter	*Oryza sativa* leaf	*Oryza sativa* seed	*Myriostachya wightiana* leaf	*Myriostachya wightiana* seed
1	Chlorophyll a (mg/gm)	17.34±0.16	----	7.85±0.03	-----
2	Chlorophyll b(mg/gm)	6.53±0.05	------	5.53±0.25	------
3	Chlorophyll t (mg/gm)	23.9±0.21	------	13.39±0.24	------
4	Reducing Sugar (mg/gm)	15±0.4	77±0.3	26.5±0.12	66±0.3
5	Non reducing Sugars (mg/gm)	71.4±10.1	635±30	445±20	540±7.5
6	Starch (mg/gm)	60±1.05	655±14	325±35	565±14
7	Protein (mg/gm)	99.2±3	71.2±3	110±4	100±10.6
8	Lipid (mg/gm)	1.41±0.18	0.381±0.18	2.435±0.18	0.128±0.00
9	Total antioxidant Activity (µM/gm)	5.76±0.18	1.71±0.12	7.83±0.12	2.97±0.12
10	Free Proline (mg/gm)	1.68±0.37	0.146±0.03	1.08±0.073	0.236±0.015
11	Nutritive Value (K.Cal/100 gm)	75.51	313.6	234.7	282.5

Each value represents the mean ± SD of three replicates
P<0.05 was considered as significant difference

Conclusion:-

The results of this study show the presence of chemical constituents with positive nutritional and medicinal properties in the seed and leaf extracts of *Myriostachya wightiana* and Oryza sativa. Our findings confirm that *Myriostachya wightiana* is a good source of protein, free proline and Antioxidants.

Acknowledgments:-

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