Quantitaive Study of Phytochemicals and Anti-oxidant Properties

The free radicals presence within the biological materials was discovered not more than 50 years (Droge, W. 2002). Due to the effect of pollution and other pathological conditions lead to stress condition (oxidative), consequently increase production of oxy radicals (Sies, H. 1996). Oxidation of other molecules may be prevented by antioxidant molecule. Free radicals are produced during oxidation reactions. This is a kind of chain reactions that leads to damage cells. Antioxidants remove the free radical by preventing the formation of intermediate in chain reactions and oxidized themselves to inhibit other oxidation reactions. So, our daily diet must be provided with antioxidant so that body defense system should well equip (Yu, et. al. 2003). This is the main reasons behind searching of sources for natural antioxidant within different types of plant materials such as fruits, vegetables, leaves, roots, oilseeds, spices etc. (Rababah, et. al. 2004). There are two materials viz. rutin and quercetin have potential antioxidant property to reduce LDL (low density lipoproteins) (DE-Whalley et. al. 1990). Rutin and quercetin directly affect on transport, metabolism by hormones regulation.

XII.A - Materials and Methods

Collection of Plant materials: The three plants *Loranthus parasiticus*, *Macrosolen cochinensis*, *Viscum album* are collected from different regions of South West Bengal. Then plant materials are preserved in freezer for further use.

Extraction and estimation of protein content of the plant: Protein was estimated through lowry method, Lowry et. al. (1951). 1 gram of nodule + 5ml of 50Mm phosphate buffer (pH- 7.5) centrifuged (10 min at 10,000rpm). The supernatant + same volume of

20% TCA, kept at 4^0C for 30 min. The precipitations are collected after centrifugation. Both TCA soluble and TCA insoluble and fractions were collected and mixed with 5ml of Lowry reagent. After 0.5 ml of Folin phenol reagent was added for a time gap of 10 min incubation and a color developed which intensity was measured by a spectrophotometer within range of 650nm after 30 min incubation.

Extraction and estimation of the sugar content of the plant: The method of Antoniew and Sprent (1978) was followed to measure total sugar within the components. 1g nodules + 5ml of 50mM K-phosphate buffer (pH-8) centrifuged (4000rpm for 15min). Then supernatant + 80% ethanol was added which is 5 times greater in volume and incubate for 4 days at 4^0C. Aquous extract was taken + 2ml of aliquote + 4ml chilled anthrone were mixed thoroughly for 15min at 90^0C in water bath. At room temperature, the testtubes were incubated and absorbance spectrum was measured at 655nm through spectrophotometer.

Extraction and estimation of the polyphenol content of the plant: The estimation of poly-phenol content was done by Folin-Ciocalteau reagent according to Px. Mallick and Singh (1980).single gram of tissue grind with 5ml ethanol (80%) and placed at centrifuged in 1000rpm for 20 minute. The supernatant was collected and kept. Again re-extraction procedure has done in previous way with same reagent and spins and pool the supernatant as above. 2ml distilled water was taken to dissolve the residue of which1ml is taken in a test tube for experiment. 2% Na_2CO_3 at a volume of 2 ml added with 1ml aliquote for 2min incubation and then add 1ml of Folin-ciocalteau reagent. The absorbance spectrum was measured at 720nm after 30min incubation in room temperature.

Extraction and estimation of the carotenoid content of the plant: Carotenoids the tetra terpenoid (c40) compounds are ubiquitus in plants (Tee and Lim, 1991). These terpenoids are oxygenated derivatives or existing as hydrocarbons (carotenes) which may be designated as accessory pigment for photosynthesis and responsible for characteristics color to different plant parts, mainly flowers and fruits. Carotenes may occur in different chemical forms have characteristics features and functions. During physiological and various pathological conditions, the levels of carotene may altered. The extraction procedure of total carotenoid content from fresh plant materials, and the separation procedure for different components from each other are given below. The total carotenoid are depends on partitioned or fraction in organic solvents depending on their solubility. The separation varies on activated magnesia of individual components which is affected by chromatography. Total carotenoid content (mainly B-carotenoid) was estimated according to the method of Kuhu and Grundmann (2007).1g plant tissue was extracted and mixed with 5ml of acetone reagent. Then centrifuged for 10min at 10,000rpm, supernatant was collected. Then 4ml aliquote was taken in a test tube for further experiment after incubation 30 min in room temperature, the colour intensity was measured by spectrophotometer at 474nm.

Extraction and estimation of the flavo**noid content of the plant**:

Flavonoid content determination was done by Aluminium chloride colorimetric method (Ghai, C.L. 2004). Each extract (0.5 ml of 1: 10 g/ml) in methanol was simultaneously mixed with 1.5 ml of methanol, 0.1 ml 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate and lastly distilled water of 2.8 ml was added. The mixture incubates at normal temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm.

The calibration curve was prepared by preparing gallic acid solutions at concentrations 12.5 to 100 µg/ml in methanol.

Catalase activity of the plant:

Catalase has a double function as it catalase the following reactions:

Decomposition of hydrogen peroxide to give water and oxygen

$$2H_2o_2 \text{ ----------} 2H_2O + O_2$$

Oxidation of H donors for example methanol, formic acid, phenol with the consumtion of one mole of peroxide

$$ROOH + AH_2 \text{----------} H_2O + ROH + A$$

The UV light absorption of hydrogen peroxide solution can be easily measured at a range of 230 and 250nm. Catalase decomposed hydrogen peroxide so that the absorption decreases with time. But this method has a restriction as the solution which do not absorb strongly at 230-250nm is not applicable.

The estimation of catalase was done according to Schroeder and Fang (1972). 1gm tissue crush with 5ml 100mM k-phosphate buffer (pH-8) and then spin with 10,000rpm for 10 min. The supernatant was collected as a enzyme extract. Then the reaction mixtures are containing enzyme Blank -800µl-2400µl Phosphate buffer +800µlEDTA. Substrate blank -400µLenzyme extract +2800µl Phosphate buffer + 800µl EDTA. Experimental Set-400µlEnz.extract+800µLH_2O_2 +2000µl Phosphate buffer + 800µlEDTA. Then take O.D. value at 240nm by spectrophotometer

Antioxidant potential of leaf By the use of stable DPPH radical:-

Radical scavenging effect of extracts in DPPH radicals:

DPPH (1,1-diphenyl-2-picrylhydrazyl) can make stable free radicals in aqueous or ethanol solution (Jung et.al. 2005). The color changes from purple to light yellow when DPPH reacts with antioxidant compound and it gives peak on at 517nm on UV-VIS spectrophotometer. The radical scavenging potential was calculated as RSC (% of inhibition).

Where, % RSC= (OD control-OD sample) / ODcontrol*100

Hydrogen peroxide Decomposition :

Determination of Decomposition H_2O_2 was done by method of Sinha12.The tested mixture contained 4 mL of H_2O_2 solution (80mM) and 5mL of phosphate buffer (pH 7.4).One milliliter of each extract dissolved in water (25mg/mL) was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature .1 mL portion of the reaction mixture was then blown into 2 ml of dichromate /acetic acid reagent at 60 s intervals .The decomposition of H_2O_2 was determined based on the standard plot for H_2O_2 was determined based on the standard plot for H_2O_2 and the monomolecular velocity constant K was determined by using the formula :

$$K=1/t\log_{10}S_0/S$$

Where, S_0 is the initial concentration and S is the final concentration of H_2O_2.

Evaluation of Trolox equivalent antioxidant capacity (TEAC):

Total antioxidant activity of the extract was measured using the TEAC assay with minor modifications (Miller et.al., 1995). The TEAC value is based on the ability of the antioxidant to scavenge the blue-green 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate).

Estimation of Rutin and Quercetin by HPLC method: Plant leaves were extracted with distilled methanol by Soxhlet apparatus. The pooled methanolic extract was evaporated under vaccume to dryness, yielding was noted. These extract was subjected for estimation of rutin and quercetin. For standard, rutin and quercetin used as a 1mg/ml solution. Chomatographic method used as a mobile phase of 0.5% formic acid: Acetonitrile in 70:30 ratios in C-18 column, flow rate 1ml/min and injected quantity is 40µl per sample.

XII.B - Result and Discussion:

The study showed a relationship between antioxidant potential and high polyphenol content. It was also declared that antioxidant potential is related to high contents of sugar and protein. The nutritional components such as sugar content is high in *Macrosolen cochinchinensis* (Leaf-0.28), but mainly sugar content comparatively high in leaf tissue. The order of sugar content are *Viscum album*> *Loranthus parasiticus*. The protein content is another aspects of nutrition of biological organism. The role of protein with in body has a specific, involvement in structural as well as defense mechanism against disease (Regina, B. 2008). Thus these three parasitic plants contained comparatively high protein content. The results of protein content shows very high amount in leaf of *Macrosolen cochinchinensis* (6.57mg) and the sequential protein content present in this plants are *Viscum album*> *Loranthus parasiticus*. Thus consumption of these plants is very suitable for children as well as younger generation as nutrition requirement for growth. Cellular damaged caused by reactive oxygen species (ROS) and causes diseases in biological organism. Thus natural

antioxidants have significance important in human health (Hazra, et.al. 2008). The plants and animals contain complex of antioxidant like polyphenol. The polyphenol content mainly present in high amount in *Macrosolen cochinchinensis* 3.12 (Leaf-3.12mg/g fresh tissue). The antioxidant potential also determined by DPPH having free radical scavenging capacity (Kato and Hirata, 1988).

The *Macrosolen cochinchinensis* is main source of antioxidant because it have the IC-50 value (89 mg) is lower than the other plants. But free radical scavenging capacity is high about 90%. But *Macrosolen cochinchinensis* shows IC-50 value (89mg) is very high indicates presence of low antioxidant activity. The HPLC assay of rutin and quercetin shows highest value in *Macrosolen cochinchinensis* to be 78% and 94% ratio w/v respectively, comparatively lower value present in *Viscum album* in both.

Table -37: Protein content mg/g fresh tissue of three hemiparasite.

SL.NO.	PLANT NAME	PROTEIN CONTENT (mg/g)
1	*Loranthus parasiticus*	5.33
2	*Macrosolen cochinchinensis*	6.57
3	*Viscum album*	6

Table – 38: Sugar content mg/g fresh tissue of three hemiparasite.

SL. NO	PLANT NAME	SUGAR CONTENT mg/g fresh tissue
1	*Loranthus parasiticus*	0.23
2	*Macrosolen cochinchinensis*	0.28
3	*Viscum album*	0.24

Table – 39: Polyphenol content mg/g fresh tissue of three hemiparasite.

SL. NO	PLANT NAME	POLYPHENOL CONTENT mg/g fresh weight
1	*Loranthus parasiticus*	3.042
2	*Macrosolen cochinchinensis*	3.12
3	*Viscum album*	1.646

Table - 40: Carotenoid content mg/g fresh tissue of three hemiparasite

SL. NO	PLANT NAME	CAROTENOID CONTENT mg/g fresh weight
1	*Loranthus parasiticus*	**2.91**
2	*Macrosolen cochinchinensis*	**3.63**
3	*Viscum album*	**2.16**

Table - 41: Flavonoid content mg/g fresh tissue of three hemiparasite

SL. NO	PLANT NAME	FLAVONOID CONTENT mg/g fresh weight
1	*Loranthus parasiticus*	**1.72**
2	*Macrosolen cochinchinensis*	**2.3**
3	*Viscum album*	**2.46**

Table - 42: Catalase content mg/g fresh tissue of three hemiparasite

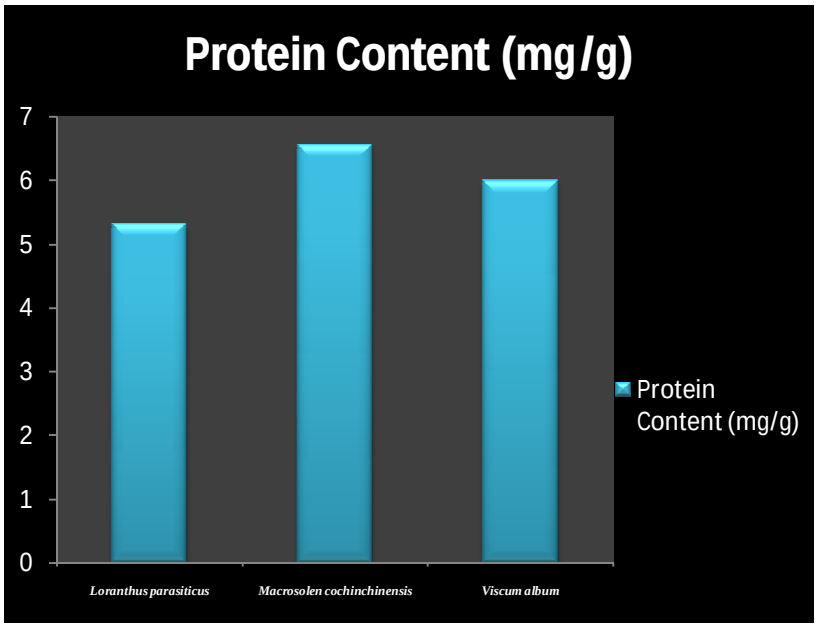
SL. NO	PLANT NAME	CATALASE CONTENT mg/g fresh weight
1	*Loranthus parasiticus*	**10.27**
2	*Macrosolen cochinchinensis*	**18.91**
3	*Viscum album*	**6.08**

Table - 43: Antioxidant potential of three hemiparasitic taxa

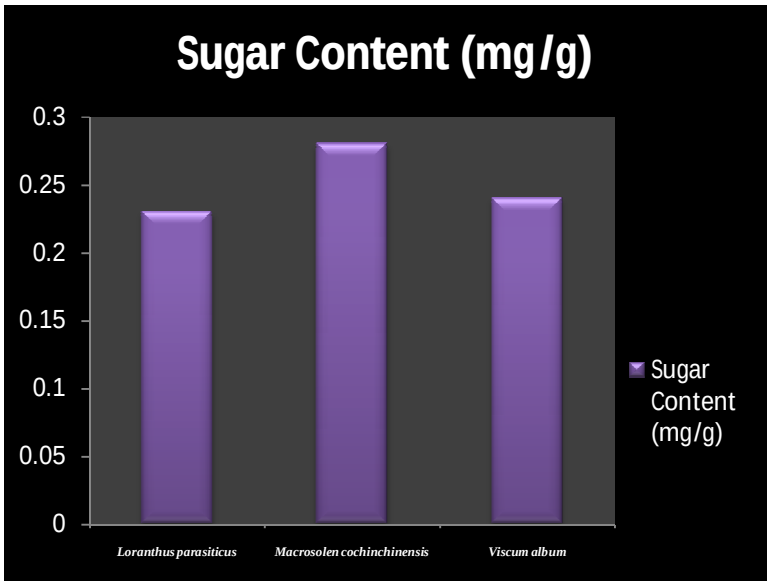
SL. NO	**PLANT NAME**	**DPPH Test (IC-50 value)**	**Hydrogen peroxide Test (IC-50 value)**	**Radical Scavenging Activity (IC-50 value)**
1	*Loranthus parasiticus*	0.74	4	0.35
2	*Macrosolen cochinchinensis*	1.05	2.6	3.2
3	*Viscum album*	0.54	3.5	1.5

Table - 44: HPLC analysis of antioxidant potential by Rutin and Quercetin

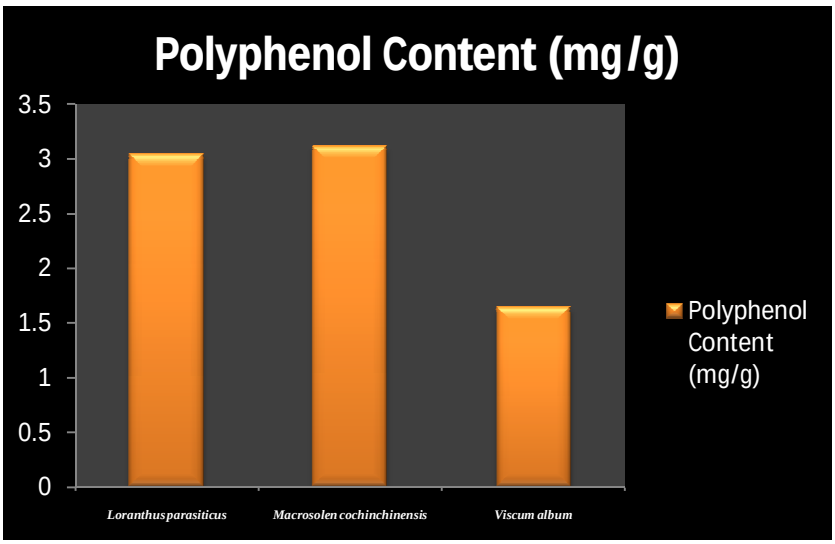
SL. NO	PLANT NAME	RUTIN ASSAY (IC-50)	QUERCETIN ASSAY (IC-50)
1	*Loranthus parasiticus*	76	92
2	*Macrosolen cochinchinensis*	78	94
3	*Viscum album*	92	56



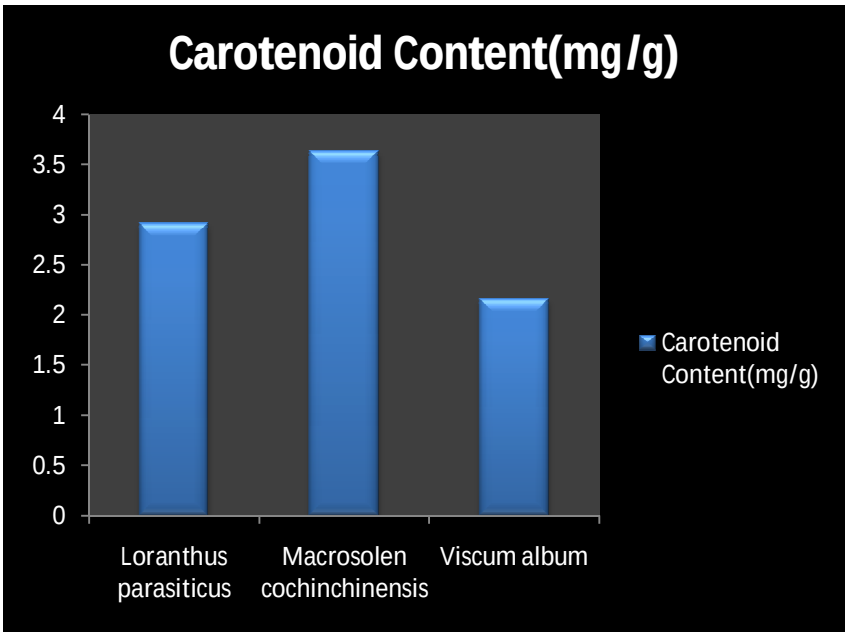
Graph – 16: Graphical representation of protein content in three parasitic plants.



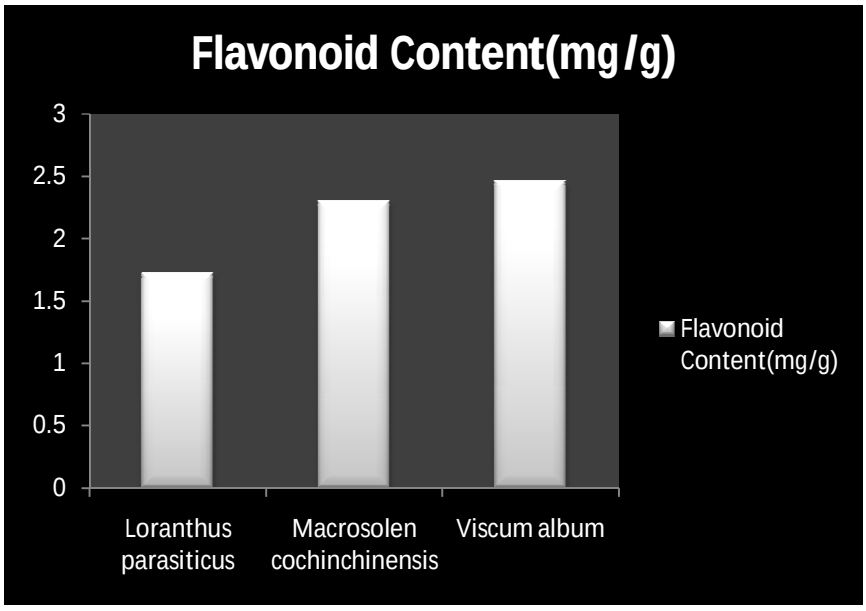
Graph – 17: Graphical representation of sugar content in three hemiparasitic plants.



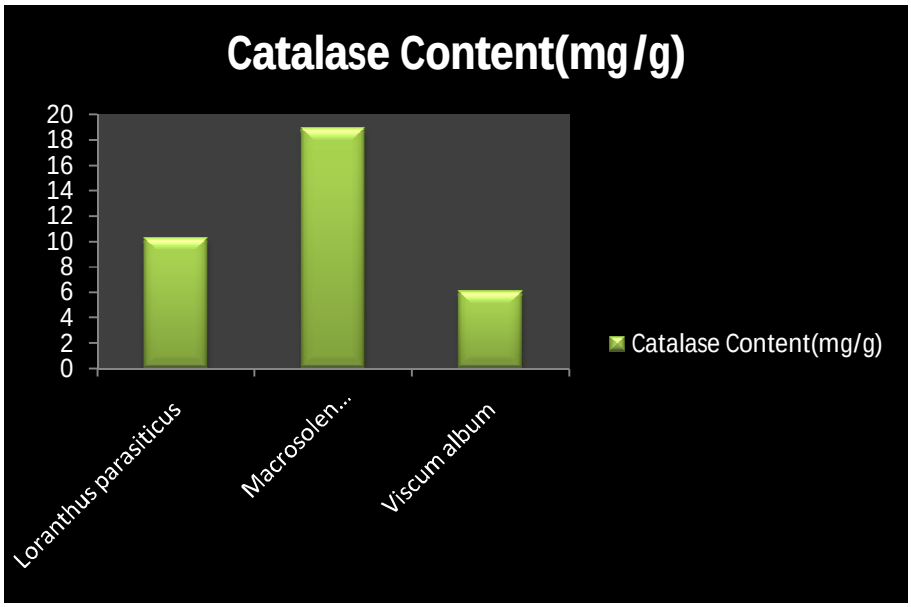
Graph – 18: Graphical representation of polyphenol content in three parasitic plants.



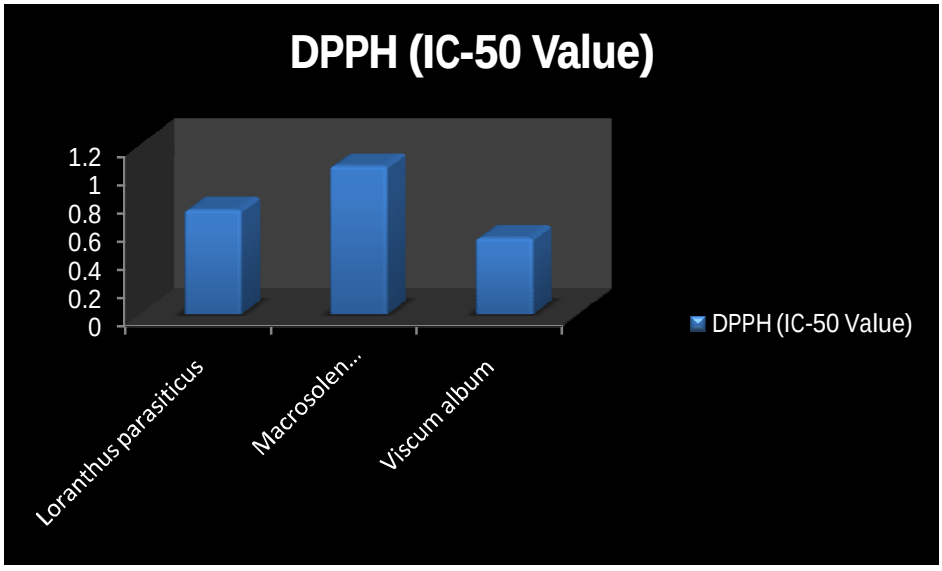
Graph – 19: Graphical representation of Carotenoid content in three parasitic plants.



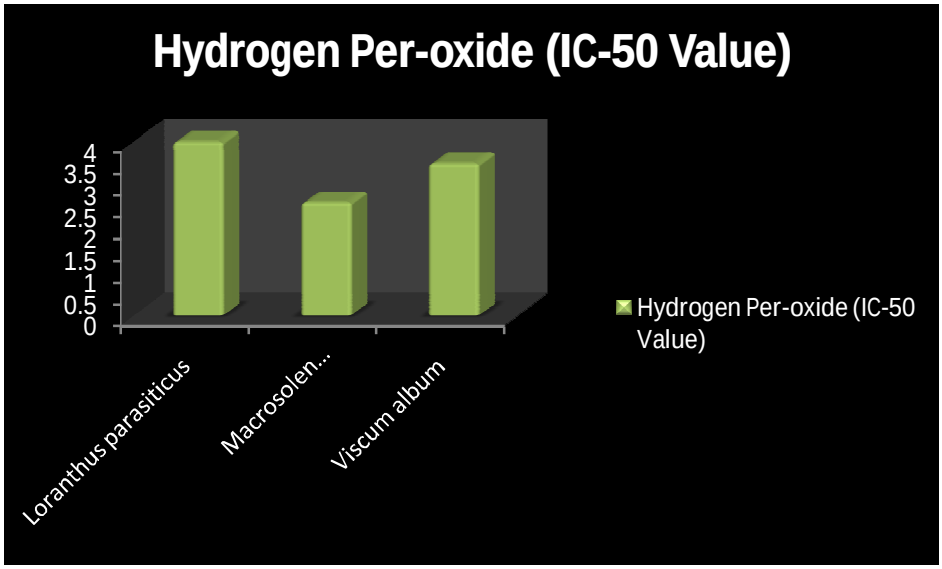
Graph – 20: Graphical representation of Flavonoid content in three parasitic plants



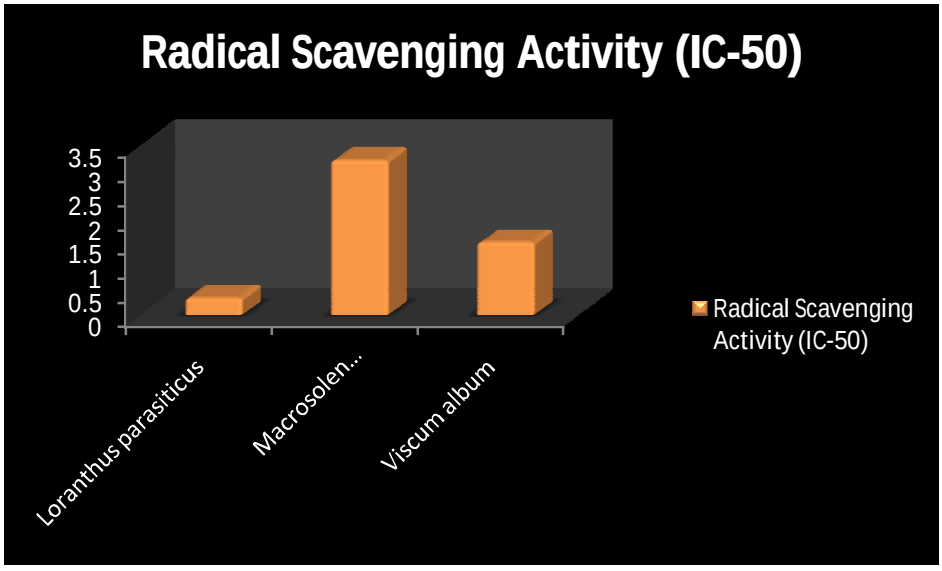
Graph – 21: Graphical representation of Catalase content in three parasitic plants



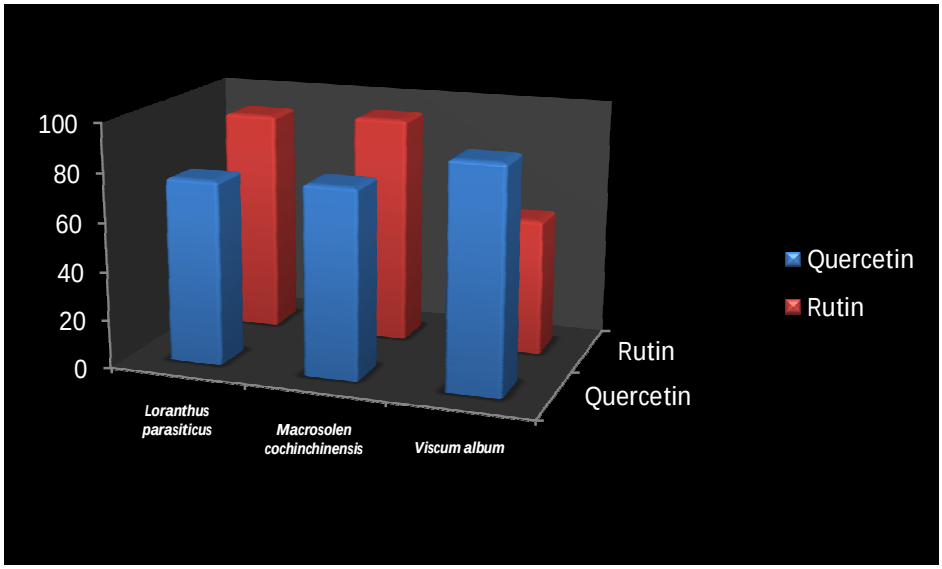
Graph – 22: Graphical representation of antioxidant potential by DPPH test



Graph – 23: Graphical representation of antioxidant potential by Hydrogen peroxide Test



Graph – 24: Graphical representation of antioxidant potential by Radical Scavenging Activity



Graph – 25: Comparative account of antioxidant potential by quercetin and rutin in HPLC method in three parasitic plants.