Results & Discussion

4.0 RESULTS AND DISCUSSION

Fruits are good sources of antioxidants that may be more effective and economical than supplements in protecting the body against oxidative damage under different conditions. An important source of antioxidants in the human diet is fruits. Fruits have taken a major part of antioxidant studies in the scientific research world (Zhang *et al.,* 2001).

Studies have shown that among the fruits, strawberry has the highest antioxidant activity followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear and honeydew melon. (Mokbel and Hashinaga, 2006). Even berries such as blueberries, blackberries, seabuckthorn or sweet rowan berries are rich in antioxidants but are not usually considered as important sources. Even fruit wastes can be used for the production of extracts possessing antioxidant activity, *e.g.* wastes from wine grape processing contains more phenolics than the unripe fruits (Pokorny, 2007).

The fruit of *Citrullus colocynthis* Linn. commonly known as 'bitter apple' has been used medicinally since ancient times. It has been suggested to possess anti-tumour activity. Phytochemical investigations have shown the bitter principle of *Citrullus colocynthis* to be cucurbitacins. The isolation and structural elucidation of a novel cucurbitacin, namely, hexanorcucurbitan glycoside in addition to three other cucurbitacin glycosides have already been done (Hatam *et al.,* 1989). The dried pulp of *Citrullus colocynthis* has been used for constipation, edema, bacterial infections, cancer and diabetes. Recently, the antioxidant effects of the aqueous extract of *Citrullus colocynthis* pulp on kidney and liver functions have been investigated (Dallak *et al.,* 2009).

The present investigation on '**A comparative study on the antioxidant properties of unripe and ripe fruits of *Citrullus colocynthis* (Linn)**' was taken up with the objective of comparing the levels of some known enzymic and non- enzymic antioxidants in the unripe fruits and the ripe fruits of *Citrullus colocynthis*. The unripe and ripe fruits (Plate I and Plate II) of *Citrullus colocynthis* were collected, powdered and extracted with water (aqueous), methanol and petroleum ether. The extracts were used for studying the enzymic, non- enzymic and free radical scavenging effects of the fruits.

The results of the study are discussed under the following headings:

4.1 ANTIOXIDANT STATUS

4.1.1 ENZYMIC ANTIOXIDANTS

4.1.1.1 Superoxide dismutase

4.1.1.2 Catalase

4.1.1.3 Peroxidase

4.1.1.4 Glutathione reductase

4.2.1 NON- ENZYMIC ANTIOXIDANTS

4.2.1.1 Total reduced glutathione

4.2.1.2 Vitamin C (Ascorbic Acid)

4.2.1.3 Carotenoids

4.2.1.4 Tocopherol

4.3 FREE RADICAL SCAVENGING ACTIVITIES

4.3.1 DPPH scavenging

4.3.2 ABTS scavenging

4.3.3 Hydroxyl radical scavenging

4.3.4 Inhibition of *in vitro* hydrogen peroxide

4.3.5 Inhibition of *in vitro* superoxide generation

4.1. ANTIOXIDANT STATUS

Evaluation of antioxidant activity of fruits, vegetables and other plant products cannot be performed accurately by any single method due to the complex nature of the phytochemicals present (Cheng *et al.*, 2008).

4.1.1 Enzymic antioxidants

Natural antioxidants manufactured in the body provide important defense against free radicals. Antioxidants prevent damage to cellular components. There are numerous enzymic antioxidant defenses designed to scavenge reactive oxygen species in the cell. These enzymes include glutathione peroxidase, catalase and superoxide dismutase. Some of these enzymes are also present in plants (James, 2010)

Table 1 and Figure 1 represent the activity of enzymic antioxidants in the unripe fruit and the ripe fruit of *Citrullus colocynthis*

4.1.1.1 Superoxide dismutase

Superoxide dismutase has been postulated as one of the most important enzymes in the enzymatic antioxidant defence system which catalyses the superoxide radicals to produce hydrogen peroxide and molecular oxygen (Sathishsekar and Subramanian, 2005).

TABLE 1

LEVELS OF ENZYMIC ANTIOXIDANT ACTIVITY

IN THE UNRIPE AND

RIPE FRUITS OF *Citrullus colocynthis*

SAMPLE	SUPEROXIDE DISMUTASE (units/g)#	CATALASE (units/g)$	PEROXIDASE (units/g)**	GLUTATHIONE REDUCTASE (units/ g)^
UNRIPE FRUIT	I.95 ± 0.85	99.17 ± 20.03	25.0 ± 4	1.47± 0.014
RIPE FRUIT	4.48 ± 0.16	276.25 ±90.16	125.0 ± 5	4.00 ± 0.51
't' Value (0.05)	2.93	1.92	22.09	4.96

Values are mean ± SD of three replicates

$ - 1 Unit is defined as Amount of enzyme required to decrease the optical density by 0.05 units at 240 nm.

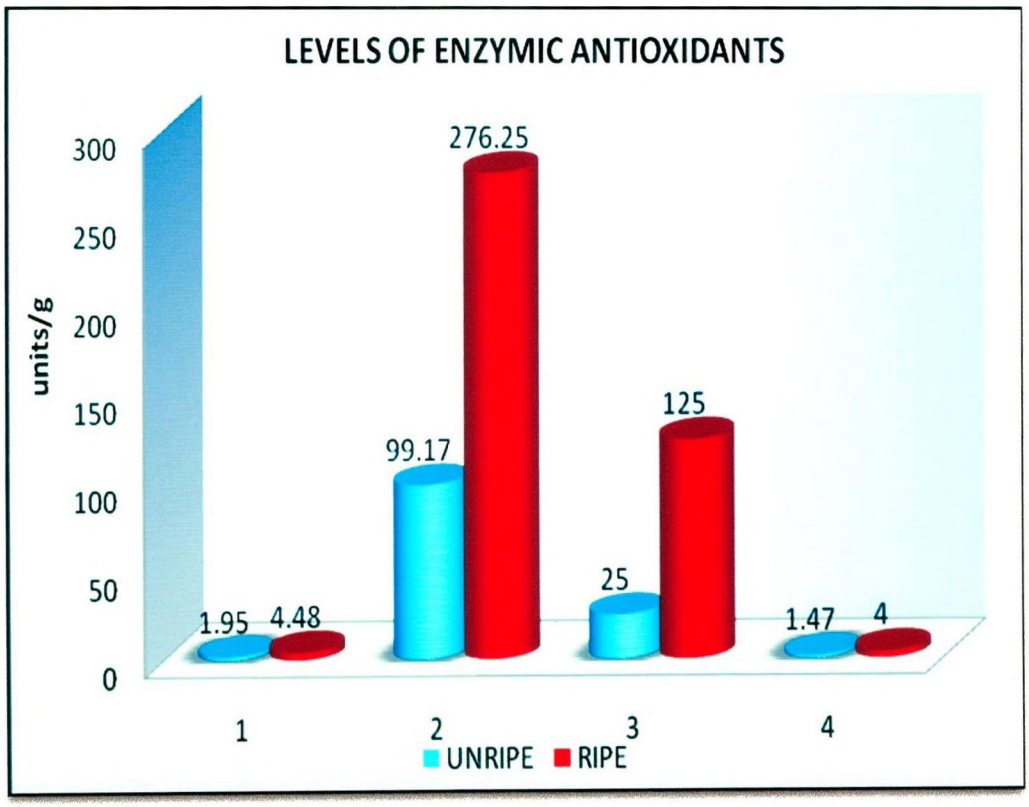
** - 1 Unit is defined as the change in absorbance/ minutes at 430nm.

- 1 Unit is defined as the amount that causes 50% reduction in the extent of NBT oxidation.

^ - 1 Unit is defined as milli moles of NADPH oxidized / m.

FIGURE 1

LEVELS OF ENZYMIC ANTIOXIDANT ACTIVITY IN THE UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



1-superoxide dismutase
2-catalase
3-peroxidase
4-gluthathione reductase

From Table 1 and Figure I, it can be inferred that superoxide dismutase was found to have a significantly higher ($p< 0.05$) activity (4.48) in the ripe fruit than the unripe fruit (1.95) of *Citrullus colocynthis*. The activity of the superoxide dismutase in the ripe fruit was approximately two and a half folds more than that of the unripe fruit.

This is in parr with the result of Laura *et al.*, 2010 who stated that the activity of superoxide dismutase showed an increasingly high value in the ripe papaya fruit of *Carica papaya* L., when compared to the early stages of the fruit which recorded a low rate of activity. This may be due to the activity of polygalacturonase and pectin methyl esterase in the ripening stage of the fruit. Another study indicated that superoxide dismutase showed a high activity in the mature leaves and fruits of Annona species compared to the unripe leaves and fruit of the plant (Baskar *et al.*, 2009). Sun *et al.*, 2010 also observed that Litchi (*Litchi chinensis* Sonn.) had an increased activity of super oxide dismutase which was observed in the pulp of the fruit when compared to the pericarp of the fruit. A similar study of *C. multistriata* showed the activity of superoxide dismutase to be higher with ripe fruit extract than the unriped fruit (James, 2010).

4.1.1.2 Catalase

Table 1 and Figure 1 indicate that of the two stages of the fruit samples analyzed, the ripe stage of *Citrullus colocynthis* showed a higher catalase activity (276.25) which was significant ($p< 0.05$) than the unripe stage (99.17). This increase was more than two and a half folds.

These observations are in agreement with the findings of Qustin, *et al.*, 2010, who inferred that catalase activity was found to be active in banana, especially in the ripe fruit. These results were obtained during a comparative study done between the fresh fruits and dry fruits at different stages of ripening. These suggest that catalase is a key enzyme in active oxygen detoxification during fruit development. The activity of catalase in ripe fruit

was also reported by Wang and Jiao, 2001 who stated that the oxidative stress increased progressively during ripening of tomatoes. There was also an increase in the enzyme during fruit ripening.

4.1.1.3 Peroxidase

Peroxidases are considered to have a potential role in fruit ripening and flavonoid biosynthesis (Singh *et al.*, 2010).

From the analysis made in the unripe and ripe fruits of *Citrullus colocynthis*, the ripe fruit was found to possess a significantly higher ($p< 0.05$) activity of peroxidase (125.0) than the unripe fruit (25.0) which had five times more activity than the unripe sample.

Another study on melon fruits explained that the peroxidase activity was correspondingly higher, which was due to the Phenylalanine Ammonia Lyase (PAL) a cytosolic protein in vascular plants, which initiates the peroxidase activity in fruits (Sen *et al.*, 2010).

Peroxidase activity was also found to be higher in yellow coloured fruits when compared to the green fruits which were determined from the total fruit extracts, especially in *Capsicum annuum* (Bell pepper), (Schantz *et al.,* 2006).

4.1.1.4 Glutathione reductase

Glutathione reductase is a key enzyme in the glutathione-ascorbate cycle and can regenerate reduced glutathione from its oxidized form. Together with glutathione, it is an important component of the ROS scavenging system in plant cells (Katerova, 2009). Glutathione reductase maintains the cellular levels which protects the cellular membranes from peroxides (Fard *et al.*, 2010).

From the values in Table 1and Figure I it can be inferred that glutathione reductase exhibited a significantly higher ($p<0.05$) activity

in the ripe fruit (4.00) than the unripe one (1.47). The ripe fruit had more than three and a half times activity of glutathione reductase than the unripe fruit.

Reports of studies conducted by Rogiers *et al*., 2007, also revealed that glutathione reductase and glutathione transferase activities rose sharply during the later stages of fruit ripening correlating well with substantial increases in the levels of both enzymes. Hence, it can be stated that glutathione-mediated free-radical scavenging system is up-regulated towards the end of ripening. Also the activity of glutathione reductase was increasing from immature fruits to stronger activity in the ripe fruit of Tunisian *Citrullus colocynthis* which was observed in acetone extracts (Marzouk *et al.,* 2009). Studies also reveal that, glutathione reductase increases with the fruit ripening process and the colour of the fruit also intensively increases during fruit maturation. Quercetin a group of plant pigments called flavonoids are largely responsible for the colours of many fruits, flowers and vegetables (Meena and Patni, 2008).

Thus from Table 1 and Figure 1 it can be concluded that superoxide dismutase, catalase, peroxidase and glutathione reductase activities were maximum in the ripe fruit when compared to the unripe fruit of *Citrullus colocynthis*

4.2.1 Non- enzymic antioxidants

The levels of non-enzymic antioxidants, namely, reduced glutathione, vitamin C, ascorbic acid, tocoperol and carotenoids in ripe and unripe fruits of *Citrullus colocynthis* are recorded in Table II and Figure 2.

TABLE II

LEVELS OF NON- ENZYMIC ANTIOXIDANT ACTIVITY IN THE UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*

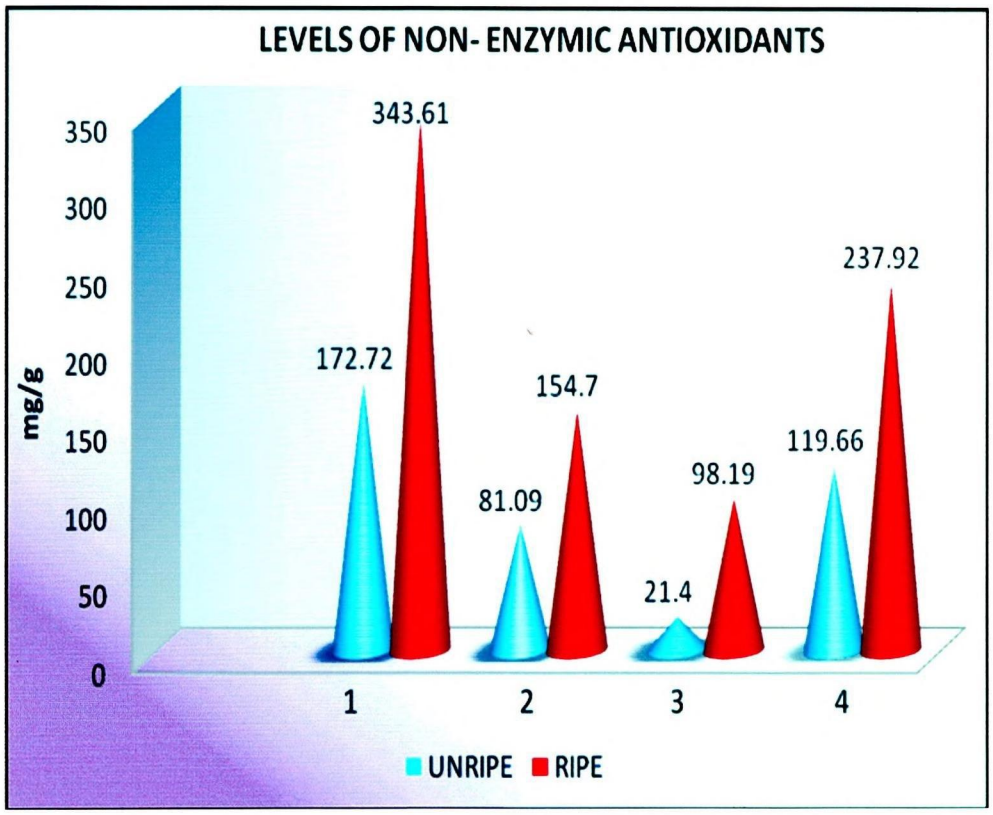
SAMPLE	REDUCED GLUTATHIONE (n mole/ g fruit)	ASCORBIC ACID (mg/g fruit)	CAROTENOIDS (mg/g fruit)	TOCOPHEROL (mg/g fruit)
UNRIPE FRUIT	**I72.72 ± 31.70**	**21.40 ± 1.11**	**119.66 ± 20.12**	**81.09 ± 8.97**
RIPE FRUIT	**343.61 ± 37.01**	**98.19 ± 11.65**	**237.92 ± 4.75**	**154.70 ± 19.09**
t Value (0.05)	**3.51**	**6.65**	**5.72**	**3.49**

Values are mean ± SD of three replicates

Significant at 5% level

FIGURE 2

LEVELS OF NON- ENZYMIC ANTIOXIDANT ACTIVITY IN THE UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



1-reduced glutathione
2-tocopherol
3-ascorbic acid
4-carotenoid

4.2.1.1 Total reduced glutathione

Glutathione is an important constituent of detoxification mechanisms operating in biological systems (Kadry *et al.,* 2010).

The levels of reduced glutathione (GSH) in the unripe and ripe fruits of *Citrullus colocynthis* are depicted in Table II and Figure 2.

It is well observed from the table and the figure that reduced glutathione in the ripe fruit was significantly ($p< 0.05$) more (343.61) than in the unripe fruit (172.72) of *Citrullus colocynthis.* The ripe fruit showed a two fold increase in the activity on comparison with the unripe fruit.

These findings are in agreement with the studies of Singh *et al.,* 2010 who reported that during the early stages of fruit ripening, tannin, flavonoids accumulate in high levels which in turn increase the glutathione in strawberry fruits and thereby increases the astringent flavour alongwith the antioxidant property.

4.2.1.2 Vitamin C (Ascorbic Acid)

Vitamin C has been found to prevent the formation of N-nitroso compounds a cancer causing compound (Guorong *et al.*, 2009).

Table II and Figure 2 reveal that the ripe fruit of *Citrullus colocynthis* had significantly higher ($p< 0.05$) amount of vitamin C content (98.19), than the unripe fruit (21.4). Vitamin C increased in the ripe fruit approximately five times more than in the unripe fruit.

This observation is in parr with the results of Bhuiyan *et al.,* 2009 who stated that, very high contents of polyphenolic substances and ascorbic acid were observed in the fruit of European cranberrybush (*Viburnum opulusedule* Marsh.) during the ripening stages of the fruit In line with the present work, reports also reveal that there is a high correlation between the ripening of fruits and the content of ascorbic acids in citrullus fruits (Rop *et al.*, 2010).

4.2.1.3 Carotenoids

Carotenoids are a major component in fruits. The major sources of vitamin A in the diet are preformed vitamin A, commonly found in foods of animal origin and provitamin – A.. The carotenoids are found in yellow and orange-fleshed fruits and vegetables and also in dark green leafy vegetables (Lopez *et al.*, 2010).

The carotenoid content in *Citrullus colocynthis,* ripe fruit (237.92) was found to be significantly higher ($p<0.05$) than that of the unripe fruit (119.66). On comparing the carotenoid content in the unripe and the ripe fruit, the ripe fruit had two times more of carotenoids content than the unripe fruit.

Supporting the above findings, another study on aqueous fractions of the tropical fruit star apple (*Chrysophyllum cainito*), surinam cherry (*Eugenia uniflora*), and jaboticaba (*Myrciaria cauliflora*) reported that high content of carotenoids was present during the fully ripened stage of the fruits (Einbonda *et al.*, 2010).

4.2.1.4 Tocopherol

Tocopherol (commonly known as vitamin E) is the most important lipophilic radical-chain-breaking antioxidant in living tissues. It also participates in the stabilization of biological membranes. The absence of vitamin E in membranes could make them highly permeable and therefore vulnerable to degradation. Vitamin E seems also to influence other important biophysical membrane characteristics such as fluidity in a manner similar to that of cholesterol (Arango and Heise, 2009).

The tocopherol content of *Citrullus colocynthis* as noticed in Table II and Figure 2 proves that the tocopherol content in the ripe fruit (154.7) was

significantly higher ($p<0.05$) than in the unripe fruit (81.09). The ripe fruit recorded a two fold increase when compared to the unripe fruit.

Thus, from Table II and Figure 2 it can be concluded that the non- enzymic antioxidants such as, reduced glutathione, vitamin C, carotenoids and tocopherol were found to be higher in the ripe fruit of *Citrullus colocynthis* when compared to the unripe fruit .

4.3 FREE RADICAL SCAVENGING ACTIVITY

The role of free-radicals in biology has become an area of intense interest. It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events in living organisms (Djeridan *et al.*, 2007). Since reactive oxygen species are important contributors to tissue injury, inflammation, cancer and many other ailments, the antioxidant properties probably contribute, at least to some extent, to the pharmacological and traditional medicinal uses of *Citrullus colocynthis* (Delazar *et al.*, 2006).

4.3.1. DPPH scavenging in the unripe fruits and the ripe fruits of *Citrullus colocynthis*

The percentage of DDPH scavenging levels in the unripe and the ripe fruits of *Citrullus colocynthis* are represented in Table III and Figure 3.

Methanol is a solvent which is capable of extracting phytochemicals having a wide polarity range. Using this solvent, it is possible to obtain a high range of plant antioxidant constituents, which could also be tested for antimicrobial systems (Gursoy and Tepe, 2009).

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of these diseases. DPPH stable free radical method

is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or extract. (Pourmorad *et al.*, 2006). Studies show that scavenging DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples, including plant extracts. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Sahgal *et al.*, 2009).

The methanol extracts as well as fractions of the plant parts were characterized by the highest DPPH radical scavenging. Methanol is frequently used to extract specific bioactive ingredients from various natural products. Anti-inflammatory ingredients have been found in the methanolic extracts of *Culcasia scadens*. A variety of solvents, pure and mixtures, have been applied to extract bioactive ingredients with various polarities. It was reported that methanol had a optimal solvent polarity for the extraction of polyphenols and other antioxidants from plant parts especially from fruits (Chon *et al.*, 2009).

Table III and Figure 3 exhibit the DPPH scavenging effect of the unripe and ripe fruits of *Citrullus colocynthis*. It can be deduced from the table and figure that the maximum percentage of inhibition was shown by the methanolic extract (90.13) of the ripe fruit, followed by in the petroleum ether extract (75.97) and aqueous extract (59.53). The difference in values between each type of extract was significant ($p< 0.05$).

Recent research reports throw light on the fact that, methanol extracts and their fraction dose increase radical scavenging activity (more than 70%) is mulberry branches, roots and leafs (Chon *et al.*, 2009). Methanolic extracts also exhibited higher antioxidant activity in various *in vitro* models compared to seed extracts. Therefore, the peel and seed fractions of fruits may potentially contain more antioxidants quantitatively or qualitatively than the pulp fractions (Guo *et al.*, 2003).

TABLE III

DPPH SCAVENGING IN EXTRACTS OF

UNRIPE AND

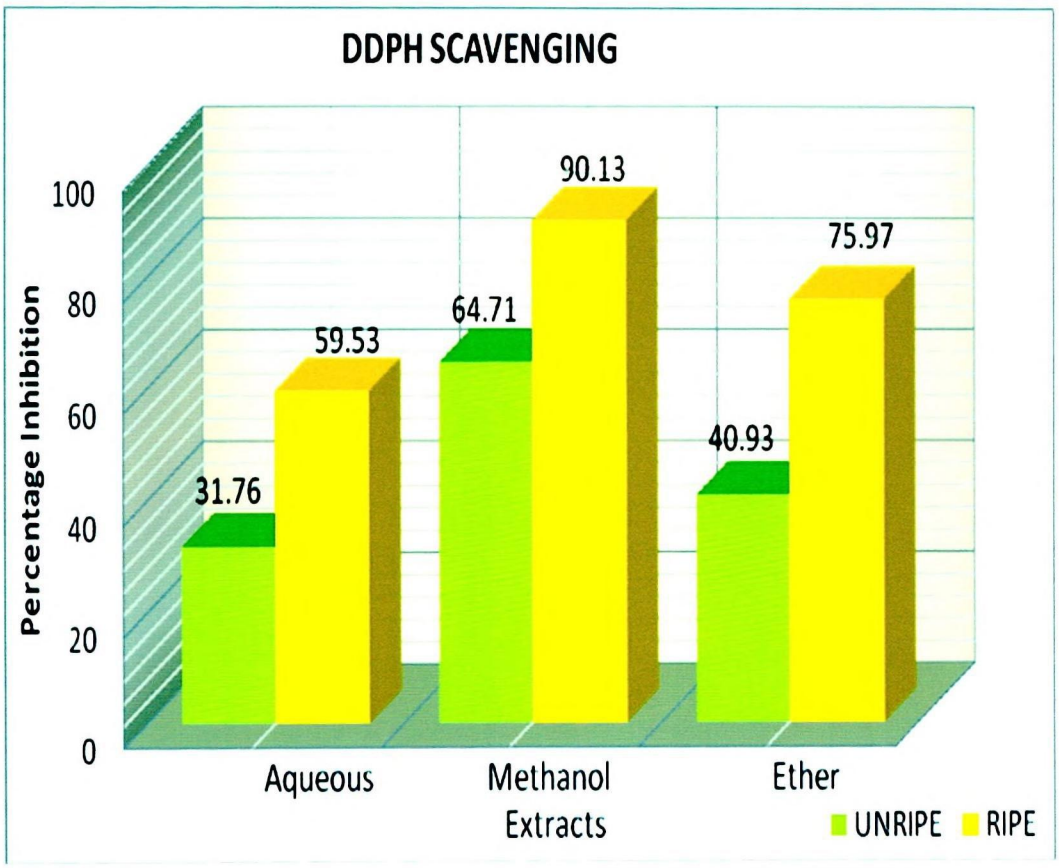
RIPE FRUITS OF *Citrullus colocynthis*

SAMPLES	PERCENTAGE INHIBITION		
	Aqueous	Methanol	Petroleum ether
UNRIPE FRUIT	31.76	64.71	40.93
RIPE FRUIT	59.53	90.13	75.97
SEd	1.29		
CD (0.05)	2.81		

Values are mean of three replicates

FIGURE 3

DPPH SCAVENGING IN EXTRACTS OF UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



Phytochemical screening revealed that the rind of *Citrullus colocynthis* and its aqueous extract contains tertiary and quaternary alkaloids, glycoside and saponin components (Hassan *et al.*, 2000). The report discussed above augmenting the antioxidant capacity determined by DPPH free radical scavenging assay of *Andrographis paniculata*, which showed that the methanolic fruit extract had higher antioxidant potential than the leaf extract antioxidant followed by stem extract (Rafat *et al.*, 2010).

4.3.2 ABTS scavenging in the unripe and the ripe fruits of *Citrullus colocynthis*

The percentage of ABTS scavenging in the ripe and unripe fruits of *Citrullus colocynthis* is presented in Table IV and Figure 4.

Antioxidant activity methods using free radicals are fast, easy and simple. The ABTS method is a very sensitive method than DPPH. The ABTS [2, 2'-azinobis (3- ethylbenzothiazoline-6-sulfonic acid)] radical cation has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents (James, 2010).

From Table IV and Figure 4 it can be mentioned that a significant ($p< 0.05$) maximum percentage of inhibition was found in the methanolic extract of both ripe (98.07) and unripe fruits (60.99) in *Citrullus colocynthis*. The petroleum ether extract of the ripe fruit showed (84.71) the second highest inhibition rate, followed by the ripe fruit (60.72) of the aqueous extract. Among the three different extracts, the aqueous extracts and the petroleum ether extract of the unripe stage of the fruits recorded the lowest inhibition.

TABLE IV

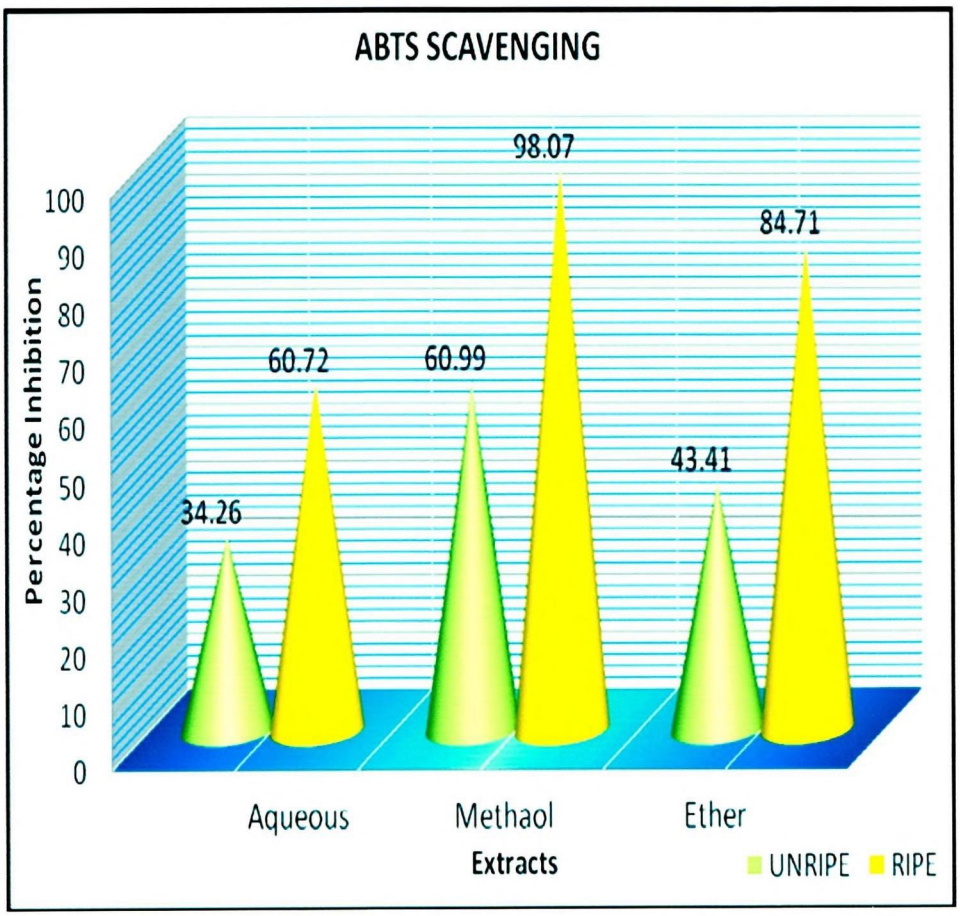
ABTS SCAVENGING IN EXTRACTS OF UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*

SAMPLES	PERCENTAGE INHIBITION		
	Aqueous	Methanol	Petroleum ether
UNRIPE FRUIT	34.26	60.99	43.41
RIPE FRUIT	60.72	98.07	84.71
SEd	1.27		
CD (0.05)	2.76		

Values are mean of three replicates

FIGURE 4

ABTS SCAVENGING IN EXTRACTS OF UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



There is a good evidence that antioxidant activity screening method in *Evax pygmaea* using ABTS radical cation decolourization assay was used .This method showed quite similar results compared to those obtained in DPPH reaction. The activity of petroleum ether fraction in ABTS test was remarkably lower where methanol fraction showed more scavenging activity (Boussaada *et al.*, 2008). It has been shown that methanol extract of *Sphaerathus indicus* showed better quenching activity in ABTS assay than the other free radical scavenging assays (Katerova, 2009).

Thus the DDPH assay and the ABTS scavenging assay showed similar scavenging results in the unripe and the ripe fruits of *Citrullus colocynthis* on comparing Tables III and IV and Figure 3 and 4.

4.3.3 Hydroxyl radical scavenging in unripe and ripe fruits of *Citrullus colocynthis*

The results of hydroxyl radical scavenging in the unripe and ripe fruits of *Citrullus colocynthis* are depicted in Table V and Figure 5.

Hydroxyl radical is an extremely reactive free radical. In addition, this radical species is considered to be one of the quick initiators of the lactoperoxidase enzyme process. This enzyme is abundant in the pulp of some few exotic fruits and in few seed species (Singh *et al.*, 2009).

The results revealed that methanol extract of the ripe fruit (91.31) had the maximum percent of scavenging followed by petroleum ether extract (77.27). Minimum scavenging effect was noticed in the aqueous extract of both the unripe fruit (25.68) and ripe fruit (26.68) of *Citrullus colocynthis*. . The scavenging effect was significantly more ($p< 0.05$) in the ripe fruit than the unripe fruit.

TABLE V

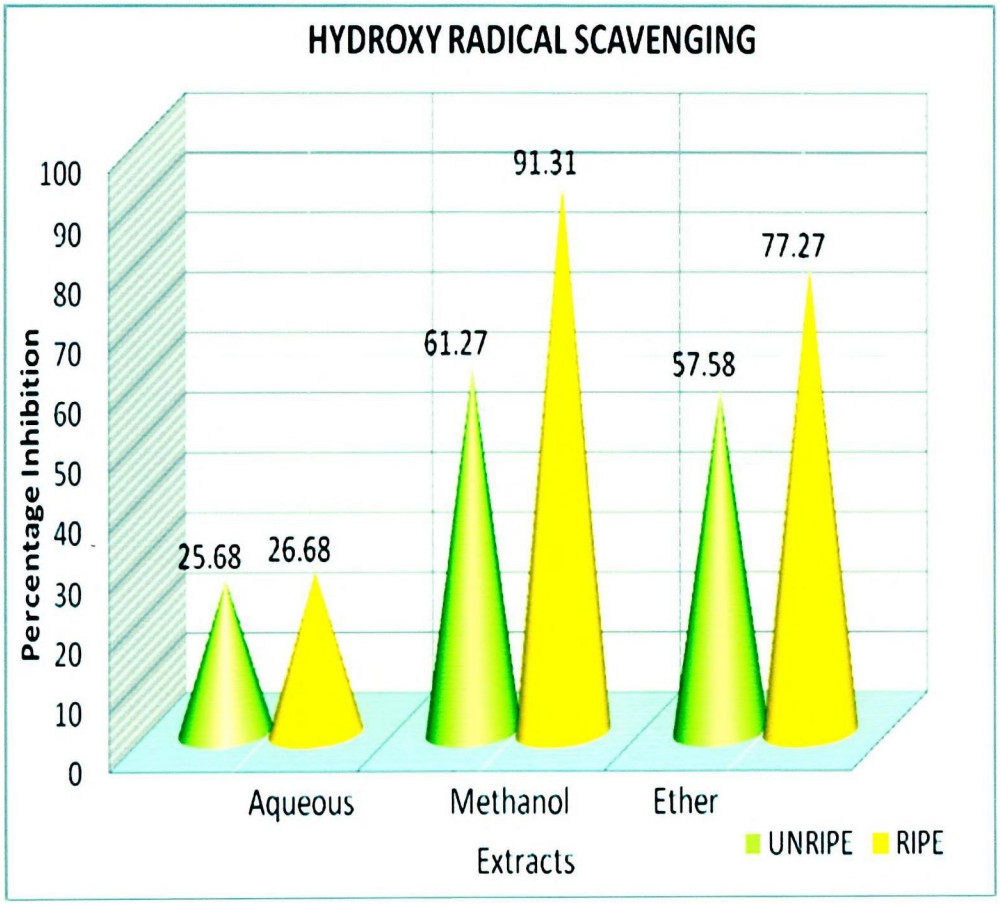
HYDROXYL RADICAL SCAVENGING IN EXTRACTS OF UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*

SAMPLES	PERCENTAGE INHIBITION		
	Aqueous	Methanol	Petroleum ether
UNRIPE FRUIT	25. 68	61. 27	57. 58
RIPE FRUIT	26. 68	91. 31	77. 27
SEd	1.33		
CD (0.05)	2.91		

Values are mean of three replicates

FIGURE 5

HYDROXYL RADICAL SCAVENGING IN EXTRACTS OF UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



The above finding is in accordance with another study which concluded that the activity of hydroxyl radical scavenging high in methanolic extract of red vine, when compared to the extract of tea which showed relatively lower scavenging activity (Toit *et al.*, 2009).

4.3.4 Inhibition of *in vitro* hydrogen peroxide generation in the unripe and ripe fruits of *Citrullus colocynthis*

The results of the studies on the inhibition of *in vitro* hydrogen peroxide generation in the unripe and ripe fruits of *Citrullus colocynthis* are recorded in Table VI and Figure 6.

In biochemical systems, hydrogen peroxide generates extremely reactive hydroxyl radicals in the presence of certain transition metal ions (e.g. iron and copper) or by ultraviolet photolysis (Okonkwo and Chinedu, 2009). Hydrogen peroxide has strong oxidizing properties. It can beformed *in vivo* by many oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds (Lee and Ng, 2007). Even many substrates and reaction systems have been applied for the study of hydrogen peroxidation and its inhibition by antioxidants. It may be noted that hydrogen peroxidation proceeds by three distinct mechanisms and the radical scavenging antioxidants are effective primarily against the free radical mediated hydrogen peroxidation (Niki, 2010).

TABLE VI

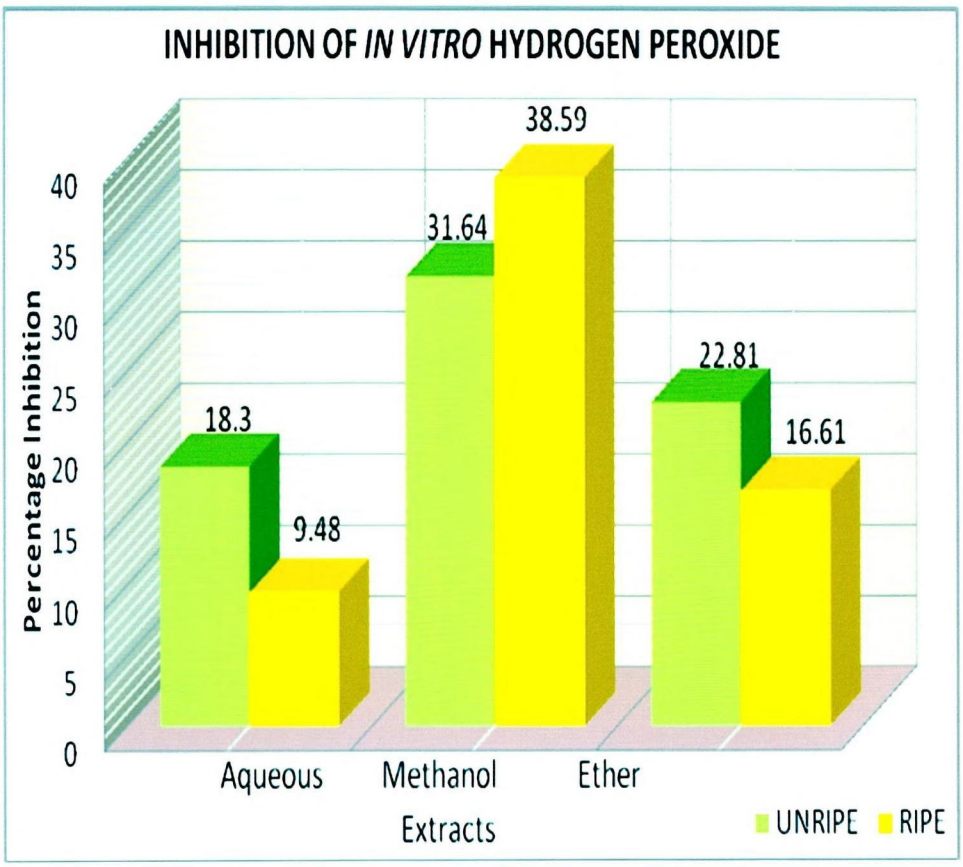
INHIBITION OF *IN VITRO* HYDROGEN PEROXIDE GENERATION IN THE UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*

SAMPLES	PERCENTAGE INHIBITION		
	Aqueous	Methanol	Petroleum ether
UNRIPE FRUIT	18. 30	31. 64	22. 81
RIPE FRUIT	9. 48	38. 59	16. 61
SEd	0.77		
CD (0.05)	1.68		

Values are mean of three replicates

FIGURE 6

INHIBITION OF *IN VITRO* HYDROGEN PEROXIDE GENERATION IN THE UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



It can be inferred from Table VI and Figure 6 that the inhibition of *in vitro* hydrogen peroxide was significantly ($p< 0.05$) high in the methanolic extract of the ripe fruit (38.59) of *Citrullus colocynthis* when compared to the inhibition in the unripe fruit (31.64). However the petroleum ether extract of the ripe fruit showed a significantly ($p< 0.05$) low inhibition (16.61) on comparison with that of unripe fruit (22.81). Similarly inhibition in the ripe fruit aqueous extract of *Citrullus colocynthis* also showed a significantly ($p< 0.05$) lower value (9.48) than the unripe fruit (18.3).

Similar to the above results the gradual increase in hydrogen peroxide inhibition during the ripening stages of banana was observed by Cheng *et al.*, 2008. He also explained that during a comparative study among the stages of banana from the unripe to the ripe (green to yellow) had a relation with hydrogen peroxide inhibition which was maximum in the ripened fruit .Similar studies also showed that the ripe guava (*Psidium guajava* L.) had high inhibition of hydrogen peroxide (Thaiponga *et al.*, 2006).

From the above table and figure it is clear that the inhibition of hydrogen peroxide generation in the methanol extract of the ripe fruit of *Citrullus colocynthis* showed the maximum inhibition followed by the unripe fruit. The lowest inhibition was observed in the aqueous extract of the ripe fruit.

4.3.5 Inhibition of *in vitro* superoxide generation in unripe and ripe fruits of *Citrullus colocynthis*

The extent of inhibition of *in vitro* super superoxide generation in unripe and ripe fruits of *Citrullus colocynthis* is presented in Table VII and Figure 7.

In superoxide inhibition the superoxide anions are generated by the oxidation of pyrogallol and the scavenging effects are expressed due to the inhibition of pyrogallol. So, any substance existing in the reaction system might have effects on the oxidation of pyrogallol (Nikkhah *et al.*, 2009).

TABLE VII

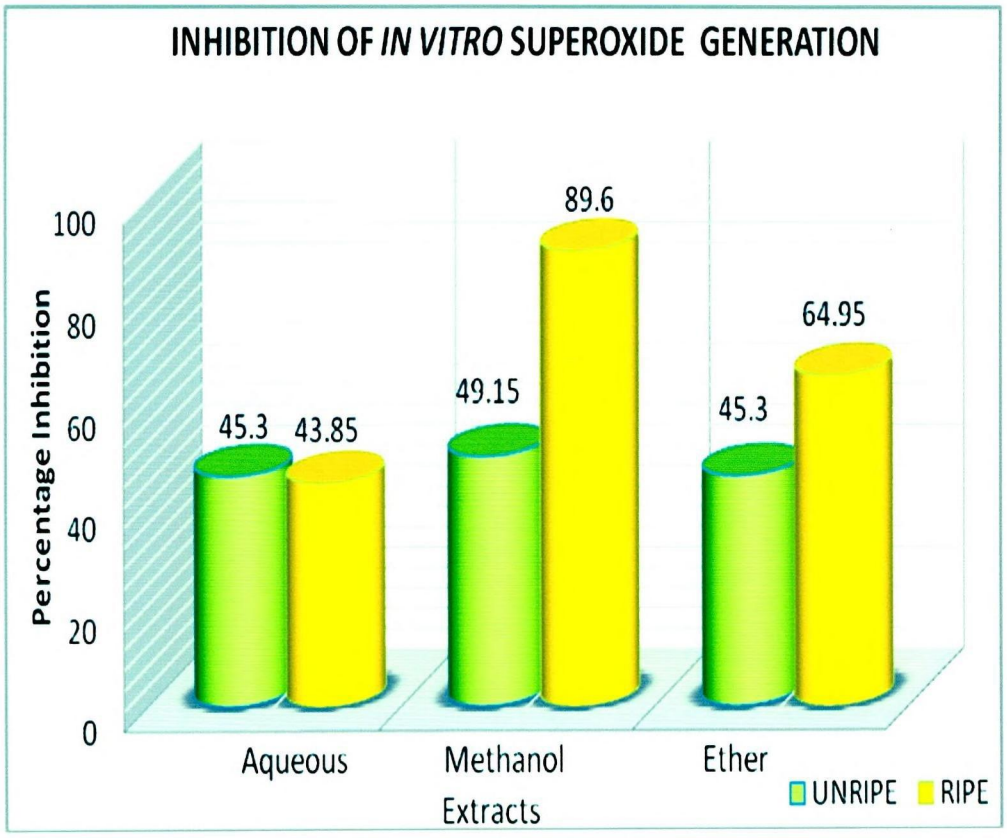
INHIBITION OF *IN VITRO* SUPEROXIDE GENERATION IN UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*

SAMPLES	PERCENTAGE INHIBITION		
	Aqueous	Methanol	Petroleum ether
UNRIPE FRUIT	45.30	49.15	45.30
RIPE FRUIT	43.85	89.60	64.95
SEd	1.30		
CD (0.05)	2.84		

Values are mean of three replicates

FIGURE 7

INHIBITION OF *IN VITRO* SUPEROXIDE GENERATION IN UNRIPE AND RIPE FRUITS OF *Citrullus colocynthis*



Superoxide anion radical has been implicated in several pathophysiological processes, due to its transformation into more reactive species such as hydroxyl radical that initiate lipid peroxidation. Superoxide has also been observed to directly initiate lipid peroxidation. Strong scavenging properties of *Ballota* species in the later stages of the fruit was on superoxide anion (Citoglu *et al.*, 2004).

The superoxide inhibition as seen in Table VII and Figure 7 showed that the superoxide was significantly high ($p< 0.05$) with maximum percentage of inhibition in the methanol extract of ripe fruit (89.6) of *Citrullus colocynthis*, followed by the petroleum ether extract of ripe fruit which recorded (64.95). The aqueous extract of the unripe fruit showed a slightly elevated inhibition (45.3) than the ripe fruit (43.85). On comparing with the three extracts a low percent of inhibition pattern was found in the unripe fruit.

In accordance with the study, the methanol extracts of barks, leaves and fruits of *Coccinia indica* plants showed effective superoxide anion radical scavenging activity especially the fruits which may serve as a potent antioxidant (Kaur and Arora, 2009). Studies suggest that the inhibition of superoxide was found to be increased during a study in the mature fruits and the mature leaves of *Pistia stratiotes* L. The reason for the high inhibition of superoxide could be due to the presence of lipid peroxidase during the ripening stages of the fruit (Sinha *et al.*, 2005).

With the results from the inhibition of *in vitro* superoxide generation, the ripe fruits of *Citrullus colocynthis* showed that the methanolic extract in the ripe fruit had the maximum inhibition, followed by the inhibition in the petroleum ether extract, whereas all the three extracts (aqueous, methanol and petroleum ether) of the unripe fruits exhibited similarly low inhibition.

As per the present investigation carried out in finding the enzymic antioxidants present in the unripe and ripe fruits of *Citrullus colocynthis,* the catalase activity was found to be maximum in the ripe fruit when compared to all the other enzymes (superoxide dismutase, catalase, peroxidase and glutathione reductase). In investigating the non-enzymic antioxidants present in the unripe and ripe fruits of *Citrullus colocynthis,* it was observed that reduced glutathione in the ripe fruit had the highest activity followed by the activity of carotenoid. It was well observed that the unripe fruits had very low non-enzymic activity.

In studying the free radical scavenging effect of the unripe and ripe fruits of *Citrullus colocynthis,* the methanolic extract of the ripe fruit in DPPH and in ABTS showed maximum scavenging effect in a similar pattern, whereas, hydrogen peroxide and the superoxide radical scavenging revealed that the methanolic extract of the ripe fruit had high elevation of scavenging, followed by petroleum ether extract and finally the aqueous extract. Therefore, with all the above results obtained from this study it could be concluded that ripe fruit of *Citrullus colocynthis* had a rich content of enzymic and non- enzymic antioxidants and thereby this fruit could be used as a good source of antioxidant.

The overall findings of the present study is summarized in the following chapter.