The life of the fruit can be conveniently divided into three major physiological stages namely growth, maturation and ripening (Wills et al., 1996). However, clear distinction between the various stages is not easily made. Growth involves cell division and subsequent cell enlargement, which accounts for the final size of the produce, while maturation only commences before growth ceases and includes different activities in different commodities and ripening is a dramatic event in the life of a fruit as it transforms a physiologically mature, but inedible plant organ into a visually attractive and taste sensation. Also ripening is said to mark the completion of fruit development (Wills et al., 1996) as it involves a series of coordinated metabolic events that alter their anatomy, biochemistry and physiology (Brady, 1987, 1992) that affect many characteristics such as colour, texture and flavour (Seymour et al., 1993) and lead to development of a soft edible fruit (Christoffersen et al., 1982).

Fruits are widely distributed in nature and one of the limiting factors that influence their economic value is the relatively short ripening period and reduced post-harvest life (Prasanna et al., 2007). Hence, determination of harvest date of fruit is an important factor for consumer’s acceptability, as quality is said to be reduced by either premature or late harvesting. Harvesting the fruits at an early stage of maturity may result in a product that has a good appearance and transports well, but yield is also said to get sacrificed with development of poor flavour. On the other hand, fruit which are harvested late may ripen quickly and perish before they are sold. Therefore, to ensure the optimum quality of fruit, there is a need for markers or indices that allow the stage of maturity to be determined with precision (Prasanna et al., 2007).

In an attempt to overcome the problems of providing healthy and nutritious food to the burgeoning population, more number of underutilized fruits, which are often neglected could be introduced for their commercial utilization (Peter, 2007). Thus, the present study deals with an integrated approach of assessing certain physiological and histological changes that occur during the growth and ripening of some underutilized fruits. The findings of the present study would be helpful in determining the maturity indices of studied fruits for ensuring their nutritional quality and adequate postharvest shelf life. Furthermore, the medicinal properties of these investigated underutilized fruits could also be understood by screening their antibacterial activity.
5:1 Histological changes

An important concept of fruit histo-architecture is that structure is not fixed, but it is in a continuous state of transition. Changes in the structure are especially important during the growth and ripening of the fruit. When an ovary develops into a fruit, the ovary wall becomes the pericarp. This pericarp may be further differentiated into three parts, more or less distinct morphologically: the exocarp or epicarp, the mesocarp, and the endocarp (Esau, 1953). Hence it is of paramount importance that the tissues and cells of the fruit are structural units in a stage of change.

According to Nitsch (1953) cell division, to certain extent, occurs during anthesis itself. However, many other researchers (e.g. Biale and Young, 1971; Bower and Cutting, 1988) observed that in most fruits, the early period of fruit growth following anthesis is characterized by rapid cell division, while subsequent growth is due to cell expansion. Furthermore, Coombe (1973); Hale and Weaver (1962) stated that when the fruit becomes an active importer of reserves or photosynthate, many of its tissues become meristematic and growth commences. i.e. the fruits in which these events are not initiated generally abscise. All these authors have expressed more or similar opinion that the ultimate size of the fruit is determined not only by cell division and cell expansion but also by cell multiplication that continues throughout the entire growth period of the fruit. It is also agreed that cell number is an important factor in fruit development and contributes to variation in fruit size.

The rapid cell divisions, occurring in both anticlinal and periclinal modes, are found to causing an increase in the number of constituent cells of the presently studied underutilized fruits from their young stage onwards and until their maturity, but subsequently the rate of cell divisions ceases. The results of the present study supports the views of Roth (1977) that the pronounced change in the shape and structure takes place, as the ovary enlarges to transform into the mature fruit, which is accompanied by localized cell division and after a specific cell size is reached, cell division is usually suspended and cell enlargement begins. Majority of the divisions occur in the mesocarpic region, while lesser or no cell divisions were observed in the epicarp and endocarp regions, once the fruit matures. In all the presently studied underutilized fruits there are gradients in the radial diameter of cells, decreasing from the centre towards the outside. These findings are in accordance with the opinion of Coombe (1976) that in most species
increase in cell volume makes by far the greatest contribution to the total expansion of the fruit, but the amount of cell enlargement is further limited by deposition of secondary wall material and hence supplementary enlargement is not possible (Sinnott, 1939).

Further, the parenchymatous cells of the presently studied underutilized fruits have also demonstrated an increase in their cell size at successive stages of fruit growth and ripening. Cleland (1971) explained reasons for this kind of increase in the cell size and according to him the amount of expansion of cells in the flesh is influenced by cell wall behaviours (plasticizing, deposition of wall material), turgor (water flux and differences in osmotic pressure inside and outside of cells) and constraints imposed upon the flesh by the extensibility of the skin or other surrounding layers. Coombe (1976) also supported this view by stating that each of these components may be influenced separately by factors such as growth regulators and the environmental factors.

The mesocarp zone, which contains edible tissues of fruit, is usually composed of parenchymatous cells, frequently of very large size at the developmental stages and during this period they are rich with cell contents. These cells possess high water content and accumulate organic substances such as sugars and organic acids. In addition to the large parenchymatous cells, the flesh of many fruits contains stone cells, which may play a role in the texture of the ripe fruits and also contributes to the taste of some fruits, since they frequently contain tannin like compounds. The mesocarpic cells of mature fruits contain abundant starch grains in the mature stage, but they decline towards ripening. However, dense cytoplasmic contents were observed until the fruit became mature but the decreased thereafter until ripening. Thus the results of the present study support the findings of Gillaspy (1993) who found structural changes in tomato fruit and concluded that the early period of slow growth is dominated by cell division whereas the rapid fruit growth period is caused entirely by cell enlargement. Coombe (1976) also stated that the weight of flesh of a ripe fruit is determined by cell number, cell volume and cell density.

The epicarps of all the presently worked out underutilized fruits are found to possess single layered epidermis with few hypodermal layers. According to Roth (1977) the epicarp is usually uniseriate and in many fruits it represents the main protective layer and sometimes includes the outer epidermis as well as some hypodermal layers. She further states that the structure of the outer epidermis of the drupe is generally similar to
Discussion

that of berries. A single layered epidermis is found in the berries of *Capparis, Eugenia, Garcinia, Maerue, Myrtus* (Sebastain, 1994) and in the drupe of *Rauvolfia tetraphylla* (Thomas and Dave, 1990), *Areca* (Sebastain, 1994), while several layered outer epidermis is found, for instance in *Amygdalus communis* (Roth, 1977), palm fruits (Reddy and Kulkarni, 1986) and *Rhus* (VonTeichman and Robbertse, 1986). Also the epicarps of the berries are said to be uniseriate to multilayered (Sebastian, 1994).

The trichomes are said to be present in the berries. However, they are found to be absent in the fruits of *Carissa carandus, Manilkara hexandra, Physalis minima* and *Syzygium cumini*, while the fruit of *Mimusops elengi* bears trichomes during its young stage but with the advancement of maturity these trichomes are found to get shed off. Similar results have been reported by Blanke (1986) in tomato fruit, where the trichomes are present in the young fruit, but at maturity they are found to be collapsed. The results of the present study are in accordance with the observation of Dave and Thomas (1991) who reported the absence of trichomes in fruit of *Carissa carandus*. Furthermore (Sebastain, 1994) has also reported the absence of trichomes in the berries of *Capparis, Eugenia, Garcinia, Maerua, Myrtus* and *Triphasia*.

The epicarpic cells are small, isodiametric or slightly elongated (tangentially) in all the underutilized fruits worked out for the present study. Roth (1977) is of the opinion that the cells of the outer epidermis of the berries are small, thick walled and isodiametric. The presence of resin canals is commonly observed in *Carissa carandus, Manilkara hexandra, Mimusops elengi* and *Syzygium cumini*. Wannan and Quinn (1990) have also reported the presence of resin canals in the members of Sapondiadeae, Rhoeae and in Anacardiaceae by VonTeichman (1990). Besides, the sclerified cells also occur in the epicarp of *Cordia dichotoma* and *Mimusops elengi*. The presence of sclerified cells have been reported in Capparis (Sebastain, 1994), *Lanea* (VonTeichman, 1987) and *Piper nigrum* (Kuriachen and Dave, 1989b). Also the tannininferous contents are found to get accumulated in *Manilkara hexandra, Mimusops elengi* and *Syzygium cumini*. According to Roth (1977) tannin cells may appear as spherical idioblasts and are of universal occurrence. Fruits of many angiosperm families such as Malvaceae (Rao, 1985) and Caesalpinaceae (Rao, 1980) are also reported to possess tanniniferous contents.
The mesocarp of *Carissa carandus, Manilkara hexandra, Mimusops elengi* and *Syzygium cumini* are multilayered, possessing small, oval to polygonal cells in the outer mesocarp and relatively larger cells in the inner mesocarp, while in *Physalis minima* the mesocarp is less differentiated. Similarly, the mesocarp of *Cordia dichotoma, Carissa carandus, Mimusops elengi* and *Physalis minima* is divided into two strata: outer and inner, which are multilayered and composed of parenchyma cells, but the presence of isolated sclereids are observed among the mesocarpic parenchyma of *Cordia dichotoma*. However, the mesocarp of *Manilkara hexandra* and *Syzygium cumini* is divided into three strata: outer, middle and inner, which are parenchymatous. The results of the present study are in accordance with the observations made by Thomas and Dave (1990) who reported the mesocarp of *Rauvolfia tetraphylla* to be composed of parenchyma cells only. Furthermore, Wannan and Quinn (1990) have also reported the mesocarp of *Dracontomelon* to be parenchymatous, but in *Spondias, Lanea, Koodersoidendron* and *Solenocarpus* sclereids are present.

Anticlinal and periclinal types of cell divisions are observed in mesocarpic cells of all the presently studied underutilized fruits. In addition, the middle mesocarpic zone of *Mimusops elengi* comprises thin walled parenchyma cells interspersed with sclereids. Similarly Roth (1977) has also reported mesocarp of *Mangifera indica* consisting of mainly parenchyma cells that constitute the edible part; vascular bundles and resin ducts. Besides, numerous tanniniferous cells are found to be present in the mesocarp of *Cordia dichotoma, Manilkara hexandra, Mimusops elengi* and *Syzygium cumini*. Also according to Nitsch (1953) tannins are generally abundant in young fruits and possibly related with respiration and metabolism of pectic substances. Soluble tannins were also observed in small scattered isolated cells of banana (Roth, 1977). Moreover tannin containing cells are of almost universal occurrence in fruit and may appear as special idioblasts or they may appear in regular parenchyma cells as in Nutmeg (Roth, 1977).

The mesocarpic cells are compactly arranged during their early growth stages, but with the advancement of growth intercellular spaces are found among them. In *Carissa carandus* the mesocarp is composed of parenchyma cells with intercellular spaces. The results of the present study are in accordance with that of Dave and Thomas (1991), who observed similar results in the fruit of *Carissa carandus*. Also according to Roth (1977) the parenchyma cells with intercellular spaces are the
characteristic of berry fruits (Berberris, Ribes, certain Solanaceae and other genera). The vascular bundles of all the fruits studied are found to occur in two rings: outer and inner strata of mesocarp, except in *Carissa carandus* where the vascular bundles occur in one stratum. Similarly, Roth and Lindorf (1971) have also observed three rings of concentric bundles in the fruit of *Coffeea arabica* and *C. canephora*. According to Sebestain (1994) each of this vascular bundle provides vascular supply to the seeds.

The endocarp of *Carissa carandus*, *Manilkara hexandra*, *Mimusops elengi*, *Physalis minima* and *Syzygium cumini* are multilayered. Concerning the topography of the composing endocarp layers and the participating meristems Roth (1977) proposed a scheme in which all possible variations are included; (a) the endocarp is represented by the inner epidermis alone or (b) the inner protoderm becomes multilayered by meristematic activity and transformed into a multilayered endocarp. The endocarps of most of the presently studied underutilized fruits fall under the second category. The inner tangential walls and about ¼ part of the radial walls of the endocarpic cells become lignified in the mature berry of piper (Kuriachen and Dave, 1989). Also during the initial stages of growth the endocarps of *Carissa carandus* is multilayered and parenchymatous, but at a later stage the inner tangential walls of the inner endocarp shows lignification, as reported earlier by Dave and Thomas (1991). Similarly the earlier reports regarding the endocarps of *Averrhoa carambola* (Dave et al., 1975), *Solanum tuberosum* (Dave et al., 1980), *Withania somniferum* (Dave et al., 1985) depicted them as multilayered and parenchymatous.

According to Roth (1977) the endocarp of drupe can be considered as the most characteristic of them, as it is very heterogenous in its origin and differentiation. In her view, the endocarp may arise only from the inner epidermis, which either remains single layered or becomes multilayered by periclinal divisions or sub-epidermal layers may be incorporated in endocarp formation. The endocarp of the presently studied *Cordia dichotoma* fruit consists of parenchymatous cells and some stony cells. Roth (1977) and VonTeichman and Robbertse (1986) have studied the fruits of members belonging to *Anacardiaceae* and *Rhoeae* and concluded that the endocarp is derived from the inner epidermis of the ovary wall. Also according to Roth (1977) the drupaceous endocarp formed by sclereids and fibers may be several layered differing from one another in cell shape.
5:2 Biochemical changes

5:2.1 pH and Total Acidity

The pH of the fruit pulp is said to be frequently below 7 and it can be as low as 3 in lemon cells. Also the total acidity of fruit is known to be due largely to the presence of organic acids, among which citric acid get accumulated in larger amounts (Ting and Attaway, 1971). Citric acid is synthesized in the mitochondria of juice cells via the Krebs cycle and is stored in the vacuole (Tucker, 1993). The fact that the organic acids are stored in the vacuole means that they are not fully available to the mitochondrial oxidation system and is thought to be the major site of their metabolism during ripening in fruits (Wills et al., 1996).

The fruits of Carissa carandus, Manilkara hexandra, Mimusops elengi, Physalis minima and Syzygium cumini worked out under the present study exhibited an increase in pH and decrease in the acidity during their successive stages of growth and ripening. Similar results have been earlier reported by Venkitakrishnan et al. (1997) in the fruit of Syzygium cumini and in the fruit of Artocarpus heterophyllus by Shamsudin et al. (2009), and they concluded it as a characteristic feature of fruit ripening. Also according to Prasanna et al. (2007) the development of taste is mainly due to decreased acidity and accumulation of sugars and organic acids. Furthermore, Wills et al. (1996) added that the change in pH may be mainly due to the leakage of organic acids from the vacuole. The decrease in the acidity according to Edmundo et al. (1998) in the fruit may be due to the utilization of acids during respiration that changes the flavour of the fruit pulp from sour to sweet. Moreover, ripening is also said to be accompanied by changes in acidity, flavor, texture, color and aroma of the fruit (White, 2002).

In contrast, the pH and acidity of the fruit pulp of Cordia dichotoma remained more or less stable at its all successive stages of growth and ripening. A similar phenomenon was also found in strawberries (Perez et al., 1997), Citrus (Hussain et al., 2004) and Persimmon (Khan et al., 2007). Baldwin et al. (2000) opined that the external factors like climate and agronomical conditions such as temperature, irrigation and fertilization regimes might also have some influence on the acidity of the fruits.
5:2.2 Pigments

Colour is the most obvious change that occurs in many fruits and is often the major criterion used by consumers to determine whether the fruit is ripe or unripe. Fruit colour or visual appearance is determined by various pigments present in the skin and flesh (Rood, 1957). As the fruit mature and ripen, green colour declines and they develop yellow, red or other colours due to pigments, which are characteristic of various fruits (Romani and Jennings, 1971). Colour changes can be either dependent or independent of ethylene action according to the pigments involved and the fruit species (Lelievre et al., 1997).

The accumulation of chlorophylls was observed increasing from young stage to the mature stage, but they decreased in the fruits of *Carissa carandus* and *Cordia dichotoma* until their ripening. Besides, the other fruits worked out under the present study namely *Manilkara hexandra*, *Mimusops elengi*, *Physalis minima* and *Syzygium cumini* exhibited an initial increase in the accumulation of chlorophylls from their young stage to the premature stage, but thereafter their chlorophyll amounts decreased to lower levels and this kind of decreased trend continued till the onset of ripening. Thus results of the present study are in line with the observations made by Seymour et al. (1993) who noticed decreasing levels of chlorophyll during the ripening of tomato fruit and stated that the precise mechanism for chlorophyll degradation is unclear, but involves both enzymatic as well as chemical reactions. Seymour et al. (1993) further explained that the solubilization of the chlorophyll into stroma may have been brought about by enzymes capable of attacking the thylakoid membranes or the chlorophyll directly, but the mechanism involved is unknown. However, according to Stanley (1998) the change in the fruit colour may be due to the synthesis of other pigments like carotenoids and/or anthocyanins, further he advocated that the loss of chlorophyll can also be mediated through several processes such as the action of enzyme chlorophyllase or enzymatic oxidation that produces low molecular weight products, which are colourless.

The amount of carotenoids in the presently investigated fruits of *Carissa carandus*, *Manilkara hexandra* and *Physalis minima* exhibited an initial increase in their content from the young stage to mature stage, but subsequently they showed decrease in the quantity with the onset of fruit ripening. In contrast the carotenoids in the fruit of
Mimusops elengi either decrease or remained unchanged in its quantity from the young stage to the mature stage, but with the onset of ripening the amount of carotenoids increased significantly. Abushita *et al.* (1996) and Giovanelli *et al.* (1999) has also reported that the pigment carotenoid accumulate late in maturation and continue its accumulation even after ripening. However, the amount of carotenoids in the fruit of Cordia dichotoma was observed to increase at its all successive stage of growth and ripening. Similar results have been reported by Rathore *et al.* (2007) in the mango and in sweet pepper by Leja *et al.* (2008).

Merzlyak *et al.* (2003) observed the pigment changes in the fruits and reasoned that during the later stages of fruit development, the tissues retain certain amounts of carotenoids or carotenoid synthesis is stimulated on the background of chlorophyll degradation. An increase in the quantity of carotenoids may also be due to the conversion of chloroplasts into chromoplasts as reported by Goodwin and Goad (1970). These authors also stated that there is a rapid synthesis of carotenoids during ripening, which is accompanied by a simultaneous decrease of chlorophyll.

In contrast, the carotenoids of the fruit of Syzygium cumini decreased to lower levels at its sequential stages of fruit growth and ripening. A similar kind of decrease in the amount of carotenoids was reported during the ripening of papaya fruit by Chen *et al.* (1996) and they noted that the loss of carotenoids did not directly accompany its chlorophyll rate, but the degradation rate of chlorophyll ‘b’ was relatively faster than that of chlorophyll ‘a’. Also according to Stanley (1998) the decrease in the carotenoids in the fruits could be due to the accumulation of other pigment anthocyanin, which gets accumulated in the vacuoles of the cells during fruit ripening.

In the developing fruit, besides chlorophylls and carotenoids which are sequestered in chloroplasts or chromoplasts, anthocyanins accumulate in the vacuoles and are responsible for change in pigmentation of the fruit (Singh and Sharma, 2000). Anthocyanins are water soluble phenolic pigments responsible for the red and blue colour of fruits. They exist as complex conjugates of the parent aglycones, the anthocyanidins and are thought to be stored in the vacuole of the cell. The colour of anthocyanins is red at acidic pH and blue at alkaline pH, but it seems unlikely that localized pH is a major factor in determining the colour of anthocyanin containing fruits (Stainley, 1998).
Anthocyanin content of the presently studied underutilized fruits is found to get accumulated at their sequential stages of growth and ripening. This increase in the amount of anthocyanins is in accordance with the results obtained by Medlicott et al. (1986) who noticed increase in anthocyanin content during the ripening of mango fruit, while similar results have been reported during the ripening of strawberry and tomato fruits by Ryozo et al. (2000) and Wang et al. (2005) respectively. Medlicott et al. (1986) also considered increase in anthocyanins as a positive attitude to plant foods. Furthermore Saure (1990), Lancaster et al. (1994) and Reay (1999) opined that the anthocyanins are the responsible molecules for the red colour of fruit. In contrast the level of anthocyanins in the fruit of Physalis minima is below detection levels, as the fruit of Physalis minima accumulated only carotenoids while the synthesis of anthocyanins was absent or limited.

5.2.3 Carbohydrates

According to Mazumdar and Majumder (2003) starch is the major storage polysaccharide found in the fruits. The conversion of starch to sugars in the fruit is an important component of the ripening process, giving the fruit its distinctive flavor as well as precursors for many of the aromatic flavor compounds. Starch is reported to get converted back to glucose by at least three different enzymes: $\alpha$-amylase, $\beta$-amylase and starch phosphorylase (Lehninger, 2005).

In the present investigation an overall decrease in the quantity of starch was noticed in the fruits of Cordia dichotoma, Manilkara hexandra, Physalis minima and Syzygium cumini, while in Mimusops elengi their levels increase from its young stage to the preripened stage, but decreased with ripening. The results of the present study supports the opinions of Mattoo et al. (1975) that starch is the major carbohydrate present in the fruits, but with the advancement of maturity, the accumulated starch is hydrolyzed into sugars, which is a characteristic event in the ripening of fruits. Similar results have been earlier reported by Lima et al. (2001) during the ripening of mango fruit. Moreover, Singh and Sharma (2000) also reported degradation of starch during the later stages of fruit growth.
The results of the present study regarding starch accumulation in the worked out fruits are in line with the results obtained by Wills et al. (1996) who explained that decrease in the starch is mainly due to the activity of primary hydrolytic enzymes. Further, Young et al. (1975) reported the activation or de novo synthesis of hydrolytic enzymes like amylase was suggested to have an active role in the degradation of starch during ripening. Besides, the decrease in the starch and increase in sugar content supports the view of Hulme (1971) who noted that the sugar levels within the fruit tend to increase progressively at all successive stages of growth and ripening.

The amount of sugars in the fruit of *Carissa carandus* initially decreased from young stage until the premature stage, but subsequently until ripening it increased. Moreover, the amount of sugars in the fruits of *Cordia dichotoma*, *Mimusops elengi* and *Manilkara hexandra* increased at their sequential stages of fruit growth and ripening, with an exception of the fruit of *Physalis minima*, which exhibited inconsistency in the sugar accumulation. Similar increase in the amount of sugars has been observed in the fruits of Strawberries (Kuti, 1992), Grapes (Manning, 1993), Opuntia (Kanellis and Roubelakis-Angelakis, 1993), Kiwi (Vishal and Neerja, 2003), Blackberry (Tosun et al., 2008) and Jackfruit (Shamsudin et al., 2009).

Recently Vishal and Neerja (2003), who reported a steady increase in the quantity of total and reducing sugars throughout the period of growth and maturation of kiwi fruit, noticed degradation of starch and increase in sugars (i.e. glucose, fructose and sucrose). Likewise, Ninio et al. (2003) also noticed increase in sugars during ripening of Koubo fruit and they opined that the increase in sugar content may be due to the breakdown of starch. Similarly Sabir et al. (2004) made more or less similar kind of observation in apple fruit and explained that the increase in sugar is chiefly due to the hydrolysis of polysaccharides. In contrast, the sugar content of the presently studied fruit of *Syzygium cumini* was found to get increased initially from the young stage to the premature stage, but its amount get decreased with the onset of ripening which may be due to the decreased activity of hydrolytic enzymes and the reduction in the import of sugars from the parent plant.
Sugars, either in free state or as derivatives, play an essential role in imparting attractive colour, flavour, appearance and texture to the fruits. The reducing sugars in the fruit of *Carissa carandus* displayed inconsistency in their amount, while the other studied underutilized fruits demonstrated an overall increase in the content of reducing sugars. An increase in the reducing sugar in the fruit, according to Sagar and Khurdiya, (1996), may be due to more rapid and partial breakdown of non-reducing sugars and other polysaccharides and their subsequent inversion to reducing sugars in the course of fruit ripening. Thus the results of present study are in agreement with the findings of Dalal *et al.* (1965) who reported that reducing sugars often increase steadily with the growth and maturation of the fruit.

However, the fruits of *Carissa carandus* and *Physalis minima* exhibited insignificant change in the amounts of their non-reducing sugars, while non-reducing sugars increased at all the successive stages of growth and ripening of the fruits of *Cordia dichotoma*, *Manilkara hexandra* and *Mimusops elengi*. In contrast, an overall degradation of non-reducing sugars was observed in the fruit of *Syzygium cumini* from its young stage until the fruit ripens. However, no sucrose was observed in the immature stage of plum fruit (Rees, 1958), but its content increased rapidly when the fruits ripened (Whiting, 1970).

### 5.2.4 Proteins, Amino Acids & Phenols

Proteins are said to be ubiquitous components of all living tissues. Although occurring in low concentration in fruits they are involved in metabolism during growth, development and ripening of fruits (Singh and Sharma, 2000). Among the presently studied underutilized fruits, *Carissa carandus* and *Physalis minima* exhibited an overall increase in the content of their proteins. Davis and Cocking (1967) reported a similar pattern in the protein content of tomato fruit. Likewise, Brady and O’Connell (1976) also demonstrated an increase in the rate of turnover of proteins in banana, while it has also been observed in many fruits by Singh and Sharma (2000). Besides, Hulme *et al.* (1968) and Dilley (1970) reported stimulated protein synthesis in the early stages of fruit development until maturation, but they noticed gradual decline towards ripening.
Tressel et al. (1975) also reported an increase in the amounts of some proteins and enzymes, while Mathooko (2000) described the dramatic increase in protein as they could be the enzymes required for the process of ripening. The results of the present study support the view of Hansen (1970) who stated that proteins are intimately concerned with all physiological events including the synthesis and degradation of proteins.

However, inconsistency in protein content was observed in the fruits of Cordia dichotoma, Manilkara hexandra, Mimusops elengi and Syzygium cumini. Similarly, the amount of protein is said to vary widely and has been previously reported by Conde et al. (2007) in the grapes. Moreover, inconsistency in the protein content is in agreement with the findings made by Gomez-Lim (1997) in several fruits. Also according to Hansen (1970) the decrease in protein content is mainly due to various physiological aspects including respiration, enzyme activity, ripening as well as the onset of senescence (Hulme, 1971). Similar kind of changes has also been reported by Sacher (1973) who reported that the quantity of proteins decreases with ripening.

The amount of amino acids in the fruits of Carissa carandus and Syzygium cumini exhibited inconsistency in their content during successive stages of growth and ripening, while the amount of amino acids remained more or less constant in the fruits of Cordia dichotoma and Mimusops elengi. Such inconsistency in free amino acids during ripening may be due to the breakdown of proteins caused by high protease activity as opined by Frankel et al. (1968). Similarly inconsistent results have been reported by Conde et al. (2007) in the developing grape.

In contrast, the amount of amino acids initially decreased in the fruits of Manilkara hexandra and Physalis minima from their young stage to the premature stage, but thereafter, until the fruit ripens it increased. The results of the present study also supports the findings of Singh and Sharma (2000) who reported decrease in amino acids and reasoned it as that as it is probably related to the process of phenol synthesis, since the phenolic compounds are the byproducts of amino acids metabolism. Similarly, a significant decline in the content of amino acids when the fruit attains maturity has been noticed by a number of workers (Ackermann et al., 1992; Sharma, 1995). Also it has been attributed that the decrease in free amino acids towards maturity and the onset of
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Ripening may be due to their incorporation into proteins required for the synthesis of various ripening related enzymes. Moreover, Rhodes (1980) opined that an increase in amino acids during ripening is a characteristic feature of fruit ripening in a number of climacteric fruits.

Phenolics represent one of the most abundant groups of compounds found in nature and are of particular interest in understanding fruit ripening as they play active role in imparting colour and flavour (Stanley, 1998). In the present study the fruits of *Carissa carandus*, *Cordia dichotoma*, *Manilkara hexandra* and *Physalis minima* exhibited insignificant change in the values of phenolic compounds at their successive stages of fruit growth and ripening. Numerous authors (e.g. Rutkowski et al., 2006; Scalzo et al., 2005; Zheng et al., 2007a) have also obtained uniform values of phenols and reasoned that the results may be due to the difference in climatic conditions, cultivation systems and harvest time. Dilley (1970) also opined that the declining trend of phenols from high levels during early growth to lower levels when the fruit attains maturity and thereafter becomes susceptible to ripening. Dilley reasoned for this kind of trend of phenols as to that the biosynthetic mechanism of certain phenolics appears to be responsive to environmental stimuli and are suspected of being involved in some types of stress responses that occurring mainly during ripening.

However, the amount of phenolics in the fruit of *Mimusops elengi* and *Syzygium cumini* was found to get a gradual fall in its quantity during successive stage of fruit growth and ripening. Similar decrease in the phenolic content has been reported in the fruits of olives by Rotondi et al. (2004); Beltran et al. (2005); Baccouri et al. (2007) and in the fruit of blackberry by Tosun et al. (2008). The findings of the present study support the view of Buren (1970), who found that the phenolic compounds declines from high levels during early growth to low level when the fruit ripens. Further Seymour et al. (1993) also attributed the astringency of fruits is due to the phenolic compounds and their decrease during ripening may be due to the formation of complex between phenolic compounds and other pectin type of cell compounds. Moreover, an increase in the phenolic content of Black plum fruit worked out currently at its early stages of growth is in accordance with the earlier report by Venkitakrishnan et al. (1997). Thus the results of the present study also supports the opinions of Kumar and Goswami (1985) that the significant accumulation of total phenols in the early stages act
as a protection mechanism to the phytohormones (auxins, gibberellins, cytokinins), which play an important role in cell division and cell enlargement. However, Kumar and Goswami (1985) have reported a declining trend of phenols from the young stage to the ripened stage.

5:2.5 Enzymatic changes

In the past several years there have been a large number of investigations on the change in enzyme activities associated with the ripening of the fruits (Sacher, 1973). According to Whiting (1970) sugar levels within the fruit tend to increase chiefly in the ripened fruit and reasoned that these changes may be due to either increased sugar importation from the plant or mobilization of starch reserves within the fruit. Furthermore Hulme (1958) reported that with the advancement of maturity, the accumulated starch is hydrolyzed into sugars, which is known as a characteristic event for fruit ripening.

During the present investigation the specific activity of amylase of all the presently worked out underutilized fruits was found to be high during their young stages, but with the advancement of growth and ripening it decreases. According to Singh and Sharma (2000) the starch degrading enzymes such as α-amylase is known to hydrolyse α (1-4) linkages of amylase at random to produce a mixture of glucose and maltose, while β-amylase attacks the penultimate linkage and releases only maltose. The results of the present study are in accordance with the results obtained by Sen et al. (1985); Lima et al. (2001) who reported similar kind of increase in the specific activity of amylase in the fruit of mangoes, but on reaching maturity their activity decreased significantly. Similarly, Fuchs et al. (1980), Tucker and Grierson (1987), Surendranathan et al. (2004) also reported increased amylase activity during ripening of many fruits. The results are in view of Leopold and Kriedemann (1975) the hydrolytic changes usually lead to formation of sugars. Initial increase in amylase activity upto maturity and thereafter decrease in the ripening of banana fruit has been reported by Omonkhua et al. (2006). However, Lima et al. (2001) opined that the climacteric rise in fruit is marked by an appreciable increase in the activity of amylase mainly due to increase in the reducing and non-reducing sugars and a simultaneous decrease in starch content.
The presently worked out underutilized fruits exhibited inconsistency at their successive sages of fruit growth and ripening in the specific activity of invertase. However, in the early stages of their fruit growth the specific activity measured high, but decreases to lower levels when the fruits ripened. The decrease in the activity of invertase has been reported by Wills et al. (1996) who opined that amylase is a primary enzyme responsible for initiating starch breakdown. Similarly Dilley (1970) also reported a more or less similar kind of increase in the specific activity of invertase in different fruits during their early growth stages, but ultimately declined as the fruits ripens, while Leopold and Kriedemann (1975) suggest that hydrolytic changes may lead to the formation of sugars required for various biochemical changes that occur when the fruit ripens. Furthermore, the level of invertase activity is observed to be related to the sucrose content of the fruits (Kuti and Galloway, 1994; Duru and Turker, 2005).

Selvaraj (1993) who noticed the activity of invertase to increase from early growth stage, but finally decreases until ripened stage of pineapple fruit opined that the increase in the invertase activity may be mainly due to the hydrolysis of sucrose. The present findings regarding the specific activities of enzymes like amylase and invertase are also in agreement with that of Venkitakrishnan et al. (1997) who reported initial increase in the activities of amylase and invertase enzymes and their subsequent decrease in the fruit of *Syzygium cumini*. Venkitakrishnan et al. (1997) also opined that this kind of variation in the activity of enzymes could be due to the accumulation of starch at the growth stages. Moreover these enzymes initially showed increased activity to form sugars, but with the advancement of maturity the activity of the enzymes decreases as the substrate decreases.

Fruit ripening has also been regarded as an oxidative phenomenon (Brenan and Frenkel, 1977), as the activities of many oxygen-detoxifying increases during this process to remove the toxic levels of H₂O₂ (Bowler et al., 1992). The specific activity of catalase in the fruits of *Carissa carandus* and *Cordia dichotoma* exhibited inconsistency, while in the *Mimusops elengi* and *Syzygium cumini* the specific activity of catalase decreases until the premature stage, but increased until ripening. The reason for this kind of inconsistency or decrease in the activity of catalase enzyme during the successive growth stages may be due to the active role of other oxidative enzymes such as peroxidase and superoxide dismutase (Brenan and Frenkel, 1977).
In contrast, the specific activity of catalase decreased until preripened stage and increased thereafter until ripening in the fruits of *Manilkara hexandra* and *Physalis minima*. Initial increase in the activity of catalase upto maturity and thereafter decreases in the ripened fruit of blackberry is reported by Wang and Jiao (2001). Similar increase in the activity of catalase has been observed by Zheng *et al.* (2007b) in the fruit of peach. Catalase is said to play an important role in removing the toxic hydrogen peroxide within the cell (DeDuve, 1983). Bowler *et al.* (1992) reported that the enzyme catalase is found predominantly in peroxisomes and also in glyoxysomes, where it functions chiefly to remove the H$_2$O$_2$ formed during photorespiration. Catalase is said to possess remarkable role in removal of electrons that lead to the production of free-radical (Abassi *et al.*, 1998). Moreover, increased activity of catalase has been reported in the fruit of mango by Mattoo and Modi (1969).

In the presently studied underutilized fruits the enzyme peroxidase exhibited inconsistency in its specific activity in the fruits of *Carissa carandus*, *Mimusops elengi* and *Syzygium cumini* at their successive stages of growth and ripening. The results of the present study suggest that the peroxidase enzyme has no role in the ripening of these fruits. The results of the present study are in accordance with the results obtained by Zauberman *et al.* (1985) in the avocado fruit. However, in the fruit of *Cordia dichotoma* the specific activity of peroxidase increases form the young stage to the premature stage and thereafter it decreased until ripening, while in the fruit of *Manilkara hexandra* the specific activity of peroxidase increases from its young stage to the preripened stage but eventually it decreases. The result of the present study support the view of Dilley (1970) who reported an increase in activity of peroxidase during the early stages of fruit development, which could be due to high production of oxidation product, but the production of oxidative products declines during the later stages due to reduction in the activity of peroxidase. Similarly, Selvaraj (1993) found the activity of peroxidase to get increased from their early stage to the preripened stage and decreased thereafter in the fruit of mango as peroxidase is considered to be a membrane bound or soluble enzyme, implicated in ethylene biosynthesis, hormonal balances, membrane integrity and respiratory control.
In contrast, the specific activity of peroxidase in the fruit of *Physalis minima* was found to get decreased from the young stage to the mature stage but later on the activity increased. Peroxidase activity is said to be under the strict control depending on the development stage and the environmental stimulus (Gadea et al., 1999). The results of the present study are in accordance with that of Ortiz et al. (2007). Moreover, the specific activity of peroxidase is said to be expressed when the plant tissue is subjected to stresses such as ripening or senescence (Grover and Sinha, 1985) as it has free radical scavenger properties (Burris, 1960). Thus, the results of the present investigation are in agreement with the view of Jimenez et al. (2002) who stated that the antioxidant system, which includes catalase, super oxide dismutase, some peroxidase and many other enzymes, plays a crucial role in the ripening process.

Most fruits soften during ripening and it is one of the major quality attribute that often dictates shelf life of the produce. According to Pilnik and Voragen (1970), Bartley and Knee (1982) and Leshem et al. (1986) the textural changes that occur during the ripening of fruit involves cell wall degradation, which consists of dissolution of the pectin rich middle lamella region.

The most noticeable change in the fruit associated with ripening is softening due to the change in cell wall biochemistry (Brady, 1987; Seymour et al., 1990). Changes in pectic component are thought to be mainly responsible for softening and this led lead to investigation of the pectic enzymes (Biale and Young, 1971) and pectic polysaccharides (Seymour et al., 1990). In the presently studied underutilized fruits an overall increase in the specific activity of polygalacturonase (PG) in the fruits of *Carissa carandus*, *Mimusops elengi* and *Syzygium cumini*. Moreover, the fruit of *Cordia dichotoma* exhibited an increase in its specific activity of PG until the preripened stage, but with the onset of ripening the activity decreases. The results of the present study are in agreement with the findings of Selvaraj (1993) who reported higher values of PG activity in certain fruits from young to the mature stage. Besides, there are several reports which indicate loss in the activity of PG in unripe fruit and increased activity of PG during ripeness of the fruit (Wong, 1995). A positive correlation between the appearance of PG and initiation of softening is shown in a number of fruits like guava (El-Zoghbi, 1994), papaya (Paull and Chan, 1983) and mango (Roe and Bruemmer, 1981). Similarly, Raju (1986) has also reported increased activity of PG to cause the solubilization of
pectic substances from the middle lamella. The solubilization of the pectic substances may occur through increase in the methylation of galacturonic acid or through a shortening of PG acid results in the breakage of calcium bridges between molecules and a loss of Ca from abscission zone.

Inconsistency in the specific activity of PG is observed for *Manilkara hexandra* and *Physalis minima*. This kind of inconsistency has been observed in the fruit of tomato by Gray *et al.* (1992) especially due to the role of enzyme glycosidases in the textural softening during ripening. Also according to Prasanna *et al.* (2007) different fruits soften at different rates and to varying degrees depending upon their inherent composition and nature. Moreover, Seymour *et al.* (1993) is of the view that the activity of PG and cellulase may be less in mature stage when compared to the ripened stage as they involve in the formation of new enzymes.

The enzyme pectinmethylesterase (PME) exhibited an increase in the specific activity in the fruits of *Carissa carandus*, *Mimusops elengi*, *Physalis minima* and *Syzygium cumini* fruits from their young stage to their preripened stage, but with the advancement of growth and ripening the specific activity decreased. Furthermore increased activity of PME has been observed in peach, tomato and pear (Tucker and Grierson, 1987). The inconsistency in the activity of PME is also reported by Reddy and Srivastava (1999) and explained the reason that temperature is said to play a crucial role in the activities of these cell wall hydrolases. PME activity was found to increase in tomatoes (Prasanna *et al*., 2007) as PME catalyses the hydrolysis of pectin methyl ester groups that results in the de-esterification of pectins. As PME is specific for galacturonide esters, its action is to remove methoxyl groups from methylated pectin (Prasanna *et al*., 2007). Moreover, PME activity has been reported to be decreased by El-Zoghbi (1994) and Prabha *et al.* (2000), while increase in its activity is noted by Selvaraj and Kumar (1989); Aina and Oladunjoye (1993) or it has been reported to remain constant by Ahmed and Lambavitch (1980); Ashraf *et al.* (1981).

In contrast, inconsistency was noted in the specific activity of PME of the fruits of *Cordia dichotoma* and *Manilkara hexandra* at their successive stages of growth and ripening. Variations in the activities of PME were reported in tomato fruit during its ripening process by Seymour *et al.* (1993). Rexova-Benkova and Markovic (1976)
Reasoned for this that the process of de-esterification proceed linearly along the chain resulting in blocks of free carboxyl groups, while, Pilnik and Voragen (1970) opined that PME preferentially attacks the methyl ester bonds of a galacturonate unit next to non-esterified galacturonate unit. Pilnik and Voragen further stated that the esterified pectic substances, make them vulnerable for PG action. Thus, the action of PME may be a prerequisite for the action of PG during ripening. Also the degradation of pectins during ripening seems to be responsible for tissue softening as reported in a number of fruits, including tomato (Tucker and Grierson, 1987), Kiwi (Redgwell et al., 1992), bush butter (Missang et al., 2001), etc.

In the present study the fruit of *Carissa carandus* exhibited an overall decrease in the specific activity of cellulase from its young stage until the fruit ripens. Similarly in *Physalis minima* the specific activity of cellulase decreased from its young stage until the premature stage, but increased during the subsequent stages until ripening. In avocado, the activity of cellulase has been reported increasing and Award and Young (1979) have suggested that cellulase may be synthesized or activated very early and play an important role in the early stages of softening. Also Hobson (1981) reported increased activity of cellulase in relation to fruit softening in avocado, peach, strawberry, tomato and papaya. Hobson has also concluded that the activities of cellulase in unripe fruits are generally low and increases dramatically during ripening.

However, inconsistency was observed in the specific activity of *Cordia dichotoma, Manilkara hexandra, Mimusops elengi* and *Syzygium cumini* at all their successive stages of fruit growth and ripening. Moreover, no cellulase activity has been reported in pears by Ahmed and Labavitch (1980). In other fruits, cellulase plays a minor role in softening. It is therefore clear that the changes occurring in the softening of fruit tissues during ripening are extremely complex. So it is perhaps not surprising that there is still doubt about the role the various enzymes play in the softening of the fruits. The presently obtained values of the specific activity of cellulase are also in agreement with the findings of Tucker (1993) who observed that the activities of cellulase may be less in the mature stage when compared with that of the ripening stage as they are thought to be involved in the synthesis of the new enzymes. Furthermore, Tucker and Grierson (1987) noticed that the major textural changes resulting during the softening of fruit are due to the enzyme mediated alterations in the structure and composition of cell wall, partial or complete solubilization of cell wall polysaccharides such as pectins and cellulose.
5:2.6 Ethylene and Respiration

Many researchers (e.g. Hamilton et al., 1990; Mattoo and White, 1991; Davies, 1995; Wilkinson et al., 1995; Bleecker and Schaller, 1996; Kieber, 1997; McGrath and Ecker, 1998) reported that the simple gas ethylene influences a diverse array of plant growth and development processes including, senescence, cell elongation and fruit ripening. Moreover, ethylene also plays an important regulatory role in the physiology of fruits (Theologis, 1993, 1994; Mathooko, 1996). Furthermore, it is generally accepted that methionine is the common precursor of ethylene throughout diverse array of plants tissue where the hormone occurs and exerts its many effects (Adam and Young, 1979).

The study of levels of ethylene in most of the underutilized fruits worked out under the present study revealed that the fruits belonged to the climacteric type, where the fruit of Carissa carandus, Cordia dichotoma, Mimusops elengi, Physalis minima and Syzygium cumini exhibited an overall increase in the levels of ethylene at all their successive growth stages, except Manilkara hexandra in which the level of ethylene decreases from its young stage to the preripened stage, but with the onset of ripening the level of ethylene increased. Similar results have been observed in many other fruits such as tomato, peach, cherry and apple (Coombe, 1976). A more or less similar kind of observation was reported in the ripening of pawpaw (Koslanund et al., 2005), coffea (Pereira et al., 2005) and banana (Johnson et al., 1997; Chillet et al., 2005).

Among the presently studied underutilized fruits, Carissa carandus, Cordia dichotoma, Mimusops elengi, Physalis minima and Syzygium cumini exhibited an overall increase in the rate of respiration at all their successive stages of growth and ripening. Similar results have been reported by Fleancu (2007) in the fruits of apple. According to Wang (1990), Lange and Kader (1997) the capacity of tissue for ethylene biosynthesis is affected by the concentration of oxygen. Kader (1986), Kanellis et al. (1993) explained that oxygen levels below those in air retard the rate of ripening and softening of climacteric fruit. All the presently studied underutilized fruits exhibit an increase in autocatalytic endogenous ethylene production and increase in respiration during the ripened stage. According to Blanke (1991) this kind of increase in ethylene and respiration are thought to be necessary to provide ATP and substrates for various anabolic processes associated with ripening of fruit.
In contrast, the rate of respiration decreased in the fruit of *Manilkara hexandra*. According to Wang (1990) high carbon dioxide concentrations inhibited the ethylene action, therefore synthesis of ripening enzymes and softening were delayed. Also according to Biale and Young (1971) the rate of respiration usually declines after harvest and remains low until the onset of ripening. Furthermore, softening of the fruit coincides with the respiratory peak.

5.2.7 Minerals

In the presently studied underutilized fruits, the amount of phosphorus was highest followed by nitrogen. Elements such as potassium, calcium and sodium were also found in moderate amounts, while copper, manganese and zinc were the trace elements to be present in the underutilized fruits investigated during the present study. All the elements present in most of the fruits studied were detected, but the element manganese was below the detection levels in the fruit of *Physalis minima*. In short, the accumulation of all the macro and microelement exhibited more or less inconsistency in deposition at successive stages of fruit growth and development. The results of the present study are in accordance with the results obtained by Barbera *et al.* (1995), Gurrieri *et al.* (2000). Similar results have also been reported by Adeyemi and Oladiji (2009) in the developing fruit of banana and Kermasha *et al.* (1987) in pineapple, while decrease in the quantity of potassium and calcium has been reported by Kamis *et al.* (2004) in the fruit of tomato.

The levels of nutrient in foods are variable. Similarly in the case of fruits and vegetables, mineral levels can be affected by various factors such as the variety of the produce item, time of harvest, ripeness, climate, soil conditions including fertilizer application, and storage and marketing conditions. Also according to Greenfield and Southgate (1992), Torelm amd Danielsson (1998) the biological materials especially the fruits and vegetables are also subject to random variation in mineral content.

5:3 Antibacterial activity

In recent years, the use of underutilized fruits is of intense interest in the food industry as an alternative to synthetic antimicrobials and other purposes. A large number of plants in different locations around the world have been extracted and used to investigate individually for their antimicrobial activity. Baris *et al.* (2006) rightly pointed out that the plant derived substances have recently become of great interest owing to their versatile applications.
Among the presently investigated underutilized fruits, the fruit of *Carissa carandus* and *Cordia dichotoma* performed less to no activity, while *Manilkara hexandra* and *Mimusops elengi* exhibited moderate activity against the tested bacterial strains. Moreover, the fruits of *Physalis minima* and *Syzygium cumini* demonstrated good to better activity against most of the bacterial strains used. Nayak (2008) who observed that the ethyl acetate root extracts of *Cordia dichotoma* exhibited good activity against SE and the results of the present study are in agreement with these, while other extracts showed moderate to no activity against all other tested organisms. Similar results have been observed by Mahida (2006) in the methanolic leaf extract of *Manilkara hexandra* against broad spectrum (Gram +ve and Gram −ve) of bacterial cultures used. Also good activity was observed by Nayak (2008) using curde extracts of *Manilkara hexandra*. Similarly Sahu *et al.* (2001) demonstrated antifungal activity against some human pathogens because of the saponin present in the *Mimusops elengi*. Also Satish *et al.* (2001) recorded significant antifungal activity against *Aspergillus* species using aqueous extract of *Mimusops elengi*.

Various extracts were screened for finding antibacterial activity of *Physalis minima* against some selected bacterial strains. Methanol extracts proved to be the best extract exhibiting high inhibition zones, followed by acetone, diethyl ether, ethyl acetate and water extract. Roychoudhury (1980) observed that the extracts of *Physalis minima* vary in the degree of their inhibition against Tobacco mosaic virus. Among the various extracts used for screening the antibacterial activity of underutilized fruits against some selected bacterial strains methanol extract exhibited good antibacterial activity against most of the tested bacterial strains. Similarly Aquil and Ahmed (2003) exhibited broad spectrum activity using crude extracts of *Syzygium cumini*.

The extracts of mature, preripened and ripened stages of all the presently studied fruits exhibited moderate to high activity against most of the organisms tested, while less or no activity was observed using the young and premature fruit extracts. Taylor *et al.* (2001) reasoned that the active compounds may be present in the extract but in insufficient quantities to show antibacterial activity, moreover the dose levels employed may also vary. Perhaps this may be the reason for not showing the MIC value in the other extracts used for this present study. Lack of activity according to
Discussion

Farnsworth (1993) can thus only be proven by using large doses. Alternatively Jager et al. (1996) has found that the active principle is present in high enough quantities and there could be other constituents exerting antagonistic effect or negating the positive effects of the bioactive agents. Furthermore Shale et al. (1999) also reported activity against the bacterial strains used and remarked that the extracts may be active against other bacterial species which were not tested.

High activity was observed in the fruit extracts of Physalis minima and Syzygium cumini, while Carissa carandus and Cordia dichotoma exhibited less activity against all the tested bacterial strains. Gram positive bacterial strains were affected more, when compared to gram negative bacterial strains. The results of the present study are in accordance with the results of Nair and Chanda (2007), Yaghoubi et al.( 2007), who observed gram negative bacterial strains as more resistant than that of gram positive bacterial strains.

A minimum inhibitory concentration is found effective using methanol extract of Physalis minima and diethyl ether extract of Syzygium cumini. Hence, the fruits of Physalis minima and Syzygium cumini possess antibacterial activity and can be further screened to find out the bioactive natural products that may serve and facilitate pharmacological studies leading to synthesis of a more potent drug with reduced toxicity. However according to Nair and Chanda (2007) the screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule and drug-like properties at the onset of drug discovery will pay off later in drug development.