Medicinal plants and herbal remedies are known to Ayurveda in India, since time immemorial. i.e history of herbal remedies is very old. Plants have served as man’s weapons against various diseases (Ayyangar and Ignacimuthu, 2005). Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of resource poor communities and the local therapy is the only means of medical treatment for such communities (Haile and Delenasesaw, 2007). About 64 % of the world population (Farnsworth, 1994 and Sindiga, 1994) and 65 % Indian population is dependant on traditional medical practice for the health care among all the existing medical practices today. As well as, Japanese, Chinese and and Korean systems of medical practice consist of plenty of medicinal plants. Various authors have mentioned about the information regarding traditional medicinal plants (Chopra et al., 1956, Rajendran and Agarawal, 2007).

The practice of traditional medicines alongwith phytomedicines and therapy designated as herbalism which is based on the applications of herbal medicinal plant extracts. The medicinal plants synthesize various aromatic and polyphenolic secondary metabolites as a phytochemicals which are essentially required for human health (Lai, 2004 and Tapsell, 2006). The system of traditional medicine is based on the knowledge of social local groups to solve their health problems. These practices are sometimes strongly related to religious belief and cultures and due to its economically affordable cost, these are applied as alternative medicines. Hence, the phenomenon of herbalism is continuously evolving. It is necessary to set up world standard research and development facilities since the flavourists and perfume experts are facing the challenging tasks of creating and developing complex compositions to meet the present and future consumer demand. It is also necessary to integrate modern knowledge with traditional knowledge.

Bone is a homeostatic and metabolically active organ receiving about 10% of cardiac output constituting important part of the skeleton (Teotia and Teotia, 2008). The osteopathy is related to the bone disorders, which is caused due to the metabolic changes, deformities and consequences of deficiencies in vitamin D and calcium content. Thus, it is essential to maintain mineralization and calcium homeostasis for better structualar integrity and health of skeleton. The various minerals like calcium, magnesium, manganese, copper, phosphorus and zinc as well as vitamin C, K and D
are essentially required for bone health. The problems of bone disorders are facing in various countries due to the side effects of synthetic drugs. To resolve these problems, the various phytomedicines and plant originated composite herbal formulations are proving beneficial effects. Thus, it is essential to screen, analyze and standardize medicinal plants. Gaikwad (2004) and Selvam (2007) speculated that the leaves of *A. cobbe* are used in the treatment of bone fractures and other ailments while the juice of the leaves is used to relieve rashes. Leaves ground with quick lime are applied to relieve stomachache. Roots are used to check diarrhoea. (Selvam, 2007). Yadav and Sardesai (2005) reported that leaves of *A. cobbe* and *A. serratus* are supposed to be useful as a medicinal and used against bone fractures. In Sri Lanka, in traditional orthopaedic treatments, the bandages prepared from bark of *A. cobbe*, peeled down towards the roots, are tied over the fractured bones or injured area (Edireeweera and Grerub, 2009). The various plant parts of *Allophylus* are used by local people from various regions of the World. *A. cobbe* and *A. serratus* are the species which are found in nearby forests of Kolhapur and its leaves are used for various medicinal purposes especially wound and fracture healing as well as young leaves as a vegetable. These situations have lead to undertake the phytochemical and pharmacological potential of the leaves, stem and root of these two medicinally important *Allophylus* species.

Some of the important findings of the present study can be summerized as follows:

**A. PHOTOSYNTHETIC PIGMENTS-CHLOROPHYLLS**

The chlorophylls are important components of primary metabolism. Chlorophyll a and b were lower in the young leaves of *A. serratus* while the level of chlorophyll a and b was elevated in mature leaves in *A. cobbe*. The chlorophyll a/b ratio was higher in leaves of *A. serratus* than that of *A. cobbe*. The total chlorophyll content in mature leaves of *A. serratus* was more than young leaves while the young leaves of *A. cobbe* showed higher total chlorophyll content.
**B. CAROTENOIDS**

Mature leaves of both species had higher level of carotenoids than younger leaves. *A. serratus* had two-fold higher values of carotenoid content than *A. cobbe*. The leaves of *A. cobbe* and *A. serratus* were rich in the total carotenoid content while the leaves of *A. serratus* exhibited higher levels of total carotenoid content which indicates that the leaves of *A. serratus* are rich source of provitamin-A carotenoids as indicated by (van Lieshout *et al.*, 2001 and Hedren *et al.*, 2000).

**C. CARBOHYDRATES**

Total carbohydrate content was higher in the stem and roots of *A. cobbe* than the *A. serratus*, the young leaves of *A. serratus* and young stem of *A. cobbe* exhibited higher level of total carbohydrates than the mature parts. Root tissue also exhibited appreciable amounts of carbohydrates. The young leaves of *A. serratus* and young stem of *A. cobbe* showed higher level of starch content than the mature one while the young stem of *A. serratus* and young leaves of *A. cobbe* exhibited lower levels of starch content. The total sugar content was slightly evaluated in mature leaves of *A. serratus* and mature leaves and mature stem of *A. cobbe* while the overall total sugar content in *A. serratus* was higher than *A. cobbe*. The reducing sugar content in young leaves of *A. serratus* and mature leaves of *A. cobbe* was higher while the mature stem of *A. serratus* and young stem of *A. cobbe* exhibited higher levels of reducing sugar. Root showed more or less similar trend of accumulation of reducing sugars. Non reducing sugars were elevated in mature leaves than young leaves in both species while its level was higher in mature stem than younger stem in *A. cobbe*. In *A. serratus*, younger stem exhibited enhanced level of non reducing sugars. The roots of both species exhibited greater level of non reducing sugars than that of younger leaves. Thus, the fractions of carbohydrates were present in appreciable quantity in leaves, stem and roots of both the *Allophylus* species which provides carbon skeleton for various types of precursors of secondary metabolites. Thus, higher levels of carbohydrates would improve the potential of bioactive compounds.

**D. TITRATABLE ACID NUMBER**

TAN values of young leaves of *A. cobbe* and *A. serratus* were two-fold greater than the mature leaves. The organic acid content in young leaves of *A. serratus* and *A. cobbe* showed higher levels than mature leaves. The young leaves of *A. cobbe* and *A.
serratus might be useful in production of certain organic acids useful for preservation which will be found beneficial for organic or chemical free preservation of food products. This will reduce the residual effects of synthetic chemicals used for food preservation.

E. ASCORBIC ACID

In *A. serratus*, mature leaves exhibited higher concentration of ascorbic acid (4.55 mg/g f.w) than that of young leaves (3.79 mg/g f.w). In *A. cobbe*, young leaves exhibited 6.64 mg/g f.w. ascorbic acid while mature leaves contained 13.39 mg/g f.w. ascorbic acid. The ascorbic acid content of mature leaves was higher than younger leaves.

F. ENZYMATIC STUDIES

1. Enzyme Catalase

The overall activity of catalase enzyme was higher in mature leaves of *A. serratus* than the *A. cobbe* while in mature leaves of *A. serratus* and *A. cobbe*, activity of enzyme catalase was higher than the young leaves. In both species, the activity of catalase increased at the maturity of leaves.

2. Enzyme Peroxidase

The mature leaves of both the species exhibited higher levels of enzyme peroxidase. In the mature leaves of *A. cobbe* and *A. serratus*, the activity of enzyme peroxidase was higher and that increase in enzyme activity was considered as the most reliable indicator of maturity and senescence (Parish, 1968). In the field observations, the leaves of *A. cobbe* are rough, choraceous and dark green and also exhibit early senescence while the leaves of *A. serratus* are light green, soft and thin. In the light of these observations, the higher level of peroxidase activity in *A. cobbe* exhibit its interspecific difference showing rough, dark, matured and senescent leaves.

3. Enzyme Polyphenol oxidase

The overall activity of enzyme polyphenol oxidase was higher in leaves of *A. serratus*. It was also noticed that the activity of PPO was elevated in mature leaves than young leaves of both species. The higher levels of polyphenol oxidase in mature
leaves might be helpful to catalyze hydroxylation of monophenols to o-diphenols exhibiting antiinsectan activity as indicated by Mayer and Harel (1981) and Duffey and Felton (1991).

4. **Enzyme Nitrate Reductase**

   The activity of enzyme NR was higher in mature leaves of *A. serratus* and young leaves of *A. cobbe* showing the better nitrogen use efficiency during early and mature stages of these two species. Thus, the assimilation of nitrates in *Allophylus* exhibited interspecific difference.

5. **Enzyme Phenylalanine ammonia lyase**

   The level of enzyme PAL was higher in the leaves of both the species which might be helpful for allocation of carbon from phenyl alanine into the biosynthesis of several other secondary bioactive molecules as indicated by Singh *et al.* (2009). Thus, the higher levels of PAL in *Allophylus* leaves exhibited further link between primary and secondary metabolism.

G. **PHYTOCHEMICAL STUDIES**

1. **Gum and mucilages**

   The young and mature leaves of *A. cobbe* and *A. serratus* exhibited higher concentration of gum and mucilages while the young stem tissue of *A. serratus* showed the moderate concentration of gum and mucilages and the stem tissue of *A. cobbe* showed absence of gum and mucilages. The root tissue of *A. serratus* and *A. cobbe* exhibited low concentration of gum and mucilages. Thus, the mucilage material extracted from leaves of *Allophylus* species in the form of suspension, gelling agents, disintegrants or might be applied in various pharmaceutical applications due to its non toxicity, low cost, free availability, emollient and non-irritating nature as indicated by Kumar *et al.* (2009b).

2. **Xanthoproteins**

   Xanthoproteins were present in all samples except mature stem of *A. serratus*. They were present in mature leaves and young stem of *A. serratus* and young and mature leaves of *A. cobbe* while moderately present in young leaves of *A. serratus*
and mature stem of *A. cobbe*. Roots of *A. serratus* and young stem and roots *A. cobbe* showed significant presence of xanthoproteins displaying antibacterial activity

3. **Phlobatannins**

The phlobatannins were detected in low concentration in young, mature leaves and stem of *A. serratus* and of *A. cobbe*. Phlobatannins were detected significantly from roots of *A. serratus* and *A. cobbe*. Phlobatannins were showing browning reactions as implicated by Szent and Vietorisz (1931) and for the synthesis of artificial melanins as indicated by Bu’lock and Harley-Mason (1951) and Beer et al (1954). Thus, roots might be useful for synthesis of such type of compounds.

4. **Coumarins**

Coumarins were very much prominent in all plant parts of *A. serratus* and *A. cobbe* exhibited pronounced levels of coumarins. Thus, the further quantitative study will help to utilize these plant parts in various cosmetics and confectionaries as a biological material.

5. **Fixed oils**

Mature leaves and stem of *A. serratus* exhibited higher levels of fixed oils while root tissue of *A. serratus* showed absence of fixed oils. In case of *A. cobbe*, leaves and young stem tissue showed absence of fixed oils whereas, mature stem and roots showed higher concentration of fixed oils. *Allophylus* leaves are traditionally used against rheumatism, joint pains as well as it is applied for healing of fractures of bones (Gaikwad, 2004 and Selvam, 2007). Thus, the leaves and stem of *Allophylus* might be useful as anti-inflammatory as well as against joint oedema and arthritis.

6. **Saponins**

Saponins were significantly detected in young leaves of *A. serratus* and both young and mature leaves of *A. cobbe* and were moderately detected from stem and root tissue of *A. serratus* and *A. cobbe*. The saponin exhibited haemolytic, antifungal, molluscicidal activity as well as it contributes as an important constituent in various herbal drugs and folk medicines exhibiting pharmacological properties as indicated by
Estrada et al. (2000). Thus, the *Allophylus* leaf and stem material might be useful in preparation of various types of herbal drugs.

7. **Cardiac glycosides**

Young and mature leaves of *A. cobbe* and *A. serratus* exhibited the low levels of cardiac glycosides while absent in stem and root tissue. Thus, the present study in *Allophylus* leaves warrants further detailed study which will be helpful for the development of drugs for heart disorders, in future.

8. **Terpenoids**

Test for terpenoid was negative in stem tissue of *A. serratus* and leaf tissue exhibited lower concentration of terpenoids and root tissue showed accumulation of terpenoids. Root, stem and leaf tissue of *A. cobbe* exhibited accumulation of terpenoids. The terpenoids indicate strong antifungal and antibacterial activity in various plant parts reported by several workers. Thus, the *Allophylus* leaves and stem with higher levels of terpenoids may serve as material to develop various antifungal, antibacterial biomolecules in near future.

9. **Total polyphenols**

Highest concentration of phenols (4.5%) was found in mature of *A. serratus* while lowest (2.0%) was found in roots of same species. Roots of both species contained comparatively lower levels of phenols as compared to stem and leaves. In *A cobbe*, younger parts represented lower concentration of phenols than mature parts, i.e. stem and leaf. The higher levels of phenolics in *Allophylus* leaves might be helpful to protect the body’s tissue against oxidative stress and beverages from *Allophylus* leaves can be implicated to prevent diseases associated with stress.

10. **Total flavonoids**

The level of flavonoid content was higher in leaves and roots of *A. serratus* while *A. cobbe* exhibited its more accumulation in young leaves. Thus, leaves of *A. cobbe* and *A. serratus* were rich in the source for flavonoids. These might be useful in the development of various bioactive compounds as well as antifungal compounds.
Further, the beverages prepared from *Allophylus* leaves may prove its antioxidant potential.

### 11. Total phytosterols

That total phytosterol content was higher in mature leaves of both the *Allophylus* species while the young leaves of *A. cobbe* exhibited rich source of phytosterols than the *A. serratus*. Mature leaves exhibit 2.5 g/100g d.w. total phytosterols which were higher than young leaves of both the *Allophylus* species. The higher levels of phytosterols in *A. serratus* and *A. cobbe* leaves further exhibited its suitability as a neutraceutical additives as indicated by Moghadasian (2000), Piironen *et al.* (2000) and Ling and Jone, (1995). The leaf juice of *Allophylus* fruit is traditionally used as beverages (Jayaweera, 1982). Thus application of phytosterols through margarine, icecreams is common practice to improve bioavailability of phytosterols while the several researchers have noticed potential positive health effects of phytosterols alongwith antioxidant and anticarcinogenic activity, the availability of phytosterols from *Allophylus* leaves might be useful in future for the designing of drugs or application of bioactive molecules through neutraceuticals.

### 12. Total tannins

In *A. cobbe*, tannin concentration was more as compared to *A. serratus*. It was also noticed that roots of *A. serratus* were rich in tannins as compared to leaves and stem. Young leaves of both species exhibited higher levels of tannin than those of mature leaves. Young stem of *A. serratus* and mature stem of *A. cobbe* exhibited higher levels of tannins. The leaves and roots of *A. serratus* exhibited 1 to 2% total tannins while the leaves, stem and roots of *A. cobbe* showed 0.8 to 1.3 % tannins in leaves, stem and root tissue. Hence, the plant parts of *Allophylus* might be utilized for preparation of chemical free mouth washes, eye washes as well as a remedy to treat rectal disorders as indicated by Hagerman and Klucher (1986). The higher level of tannin content might be contributing antioxidant potential of these plant parts.

### 13. Total alkaloids

In both species, mature leaves exhibited higher concentration of alkaloids than young leaves. Mature stem of *A. serratus* showed higher levels of the alkaloids than
that of young stem. In *A. cobbe*, young stem exhibited higher levels of alkaloids than mature stem. Roots of *A. cobbe* showed lowest alkaloid content among all parts of two species. Alkaloid content in stem tissue was higher than leaf and root tissue while the mature leaves of *A. serratus* and *A. cobbe* also exhibited the higher levels of alkaloids. Thus, the root and stem tissue of *A. serratus* and leaf and stem tissue of *A. cobbe* might be useful for the extraction of alkaloids. Further detailed study, will throw light on this aspect.

**H. GCMS ANALYSIS OF YOUNG LEAVES OF A.COBBE AND A.SERRATUS**

It is observed that the leaves of both the species were rich in terpenes, alkanes, alcohols, esters and fatty acids. The major compound found in *A. cobbe* was phytol while the major compound observed in *A. serratus* was 3-methyl butanol. Phytol was found common in both species. The abundant presence of phytol in leaves of *Allophylus* species suggests that these species may prove helpful in prevention of degenerative diseases. As well as, phytol is used in artificial synthesis of vitamin E and vitamin K. Additionally, phytol has various applications in cosmetics and fragrance industry suggests the application of these herbs in cosmetics, which may prove a cost effective alternative to current synthetic chemicals used in cosmetic industry. Thus, the further detailed studies will ascertain phyto pharmaceutical activity of these medicinal species. In addition, the isolation and purification of identified bioactive compounds from *Allophylus* may be useful to formulate the novel drugs for various diseases.

**I. MINERAL NUTRITION**

**1. Calcium**

The calcium content in young leaves of *A. serratus* was higher than the mature leaves while the mature leaves of *A. cobbe* exhibited higher level of calcium content than young leaves. The calcium content in mature stem of *A. serratus* was higher than the young stem whereas in *A. cobbe*, young stem showed higher level of calcium than mature stem. Roots of *A. serratus* exhibited higher level of calcium than roots of *A. cobbe*. Leaves of *A. cobbe* and *A. serratus* are used by traditional healers for various bone disorders. Ca is well known to enhance the qualities of bones and teeth, coagulation of blood, functioning of central nervous system, milk production, and
transmission of electrical and chemical messages and absorption of vitamin B12 from digestive tract. The leaves of *Allophylus* showed higher level of calcium which might be used in functioning of various metabolic reactions.

2. **Magnesium**

The magnesium content in young and mature leaves, young and mature stem and roots exhibited more or less uniform pattern of accumulation in *A. serratus* and *A. cobbe* except mature leaves of *A. cobbe* exhibited 3-fold higher level of magnesium than the young leaves. As Mg is involved in various types of muscular and neuromuscular defects as indicated by Shils (1969), thus the optimum level of magnesium of *A. serratus* and *A. cobbe* may certainly prove beneficial if it is consumed in the form of extract or beverage.

3. **Iron**

The iron content of young leaves and young stem was higher than the mature ones in *A. serratus* whereas *A. cobbe* young leaves exhibited ten-fold higher level of iron than the mature leaves. On the contrary, mature stem of *A. cobbe* exhibited two-fold higher level of iron than mature stem. The roots of *A. serratus* showed two-fold higher level of iron than the *A. cobbe* roots. Thus, the higher level of iron content in young leaves and mature stem of *A. cobbe* might be useful to fulfill the disorders caused due to iron deficiency.

4. **Copper**

The copper content was negligible in *A. serratus* plant parts while the *A. cobbe* young stem exhibited higher levels of copper but was not detected in the roots of *A. cobbe* showed higher levels of copper. It was also evident that copper was accumulated in shoot tissue than the roots of both the *Allophylus* species. Thus, the leaves of *A. serratus* and *A. cobbe* containing moderate levels of copper might prove safe when these leaves are used as crude drugs except the young stem of *A. cobbe* which will need further investigations.

5. **Zinc**

Zinc content in young leaves of *A. serratus* was 8-fold higher than mature leaves of *A. serratus*. As Zn plays important role in influencing immune system, the
leaves of both *A. cobbe* and *A. serratus* may be used for Zn supplement and reducing susceptibility of diseases and improve immune power. Thus, the leaves of *Allophylus* can be employed in the herbal preparations to cure bone disorders, rheumatism and immune power.

**6. Manganese**

The manganese content was higher in mature leaves of *A. serratus* while young leaves of *A. cobbe*. On the other hand, mature stem of *A. cobbe* exhibited higher manganese content than young stem and young stem of *A. serratus* showed higher levels of manganese than the mature stem. The roots of *A. serratus* showed two-fold higher level of manganese than *A. cobbe*. The deficiency of Mn disturbs the metabolism of carbohydrates exhibiting skeletal abnormalities and impaired growth whereas the mild toxicity of Mn can cause vascular pains and severe toxicity leads to develop the symptoms similar to Parkinson’s disease as indicated by Chandra (1983). Thus, the lower levels of Mn in *Allophylus* leaves would certainly improve the quality of drug when the leaves and stem of *Allophylus* are recommended as a phytomedicine.

**7. Cobalt**

The cobalt content in roots and stem of *A. serratus* was considerably higher than the *A. cobbe*. It is also noticed that the leaf tissue of both the *Allophylus* species exhibited higher levels of cobalt than the stem tissue. The stem tissue of *A. cobbe* exhibited negligible amount of cobalt and root tissue exhibited absence of cobalt which will be found useful and safe if the *Allophylus* is used in herbal preparations.

**8. Chromium**

Overall chromium content in *A. serratus* plant parts was higher than the *A. cobbe*. The mature leaves and mature stem of both the species exhibited slight difference in the level of chromium content. The root tissue of *A. serratus* exhibited considerably higher level of chromium than the root tissue of *A. cobbe*. It is also noticed that, in *A. cobbe*, Cr content was significantly accumulated in shoot tissue than the root tissue. The heavy metal Cr is toxic to the human beings in the form of Cr VI. Cr content in plants is in the form of Cr III. Hence, the material of *A. cobbe* can safely used in preparation of herbal drugs.
9. Cadmium

Cadmium content was not detected from root, stem and leaves of *A. serratus* while the leaves, stem and roots of *A. cobbe* exhibited low levels of cadmium. Thus, due to low levels of cadmium in *Allophylus*, the leaves and stem of these plants can be safely used in the preparation of various herbal medicines.

J. ANTIOXIDANT POTENTIAL

1. DPPH radical scavenging activity

Mature leaves of both species have higher DPPH radical scavenging activity as compared to young leaves. Mature stem of both species exhibited higher level of DPPH radical scavenging activity than that of young stem. Roots of both species had near about equal activity. Maximum scavenging activity was exhibited by mature leaves of *A. cobbe*. The *Allophylus* plant parts of both the species exhibited antioxidant potential which can be used to prevent cancer chemoprotective effects, as well as arthritis caused due to oxidative damage.

2. Metal chelating ability

The mature leaves and mature stem of both species showed greater metal chelating ability as compared to their young leaves and young stem. Mature leaves of *A. serratus* exhibited high metal chelating activity than the *A. cobbe*. It is also evident that the overall metal chelating ability of *A. serratus* species was higher than the *A. cobbe*. The mature leaves of *Allophylus* exhibited higher metal chelating ability than the young leaves because the aqueous extracts obtained from mature leaves of *A. serratus* and *A. cobbe* exhibited most active extract interfered with the formation of ferrous and ferrozine complex indicating that it has higher chelating activity and captures ferrous ions before ferrozine than the mature leaves and stem. Hence, the metal chelating ability of *Allophylus* plant parts can be potentially used in food industry or to treat against the neurodegenerative/aging diseases in future.
K. BIOMEDICAL EVALUATION

1. Antibacterial activity

It was observed that Cefotaxime showed 14.75mm zone of inhibition against B. subtilis while the aqueous and ethanolic extracts of mature leaves of A. serratus showed 19mm and 17mm maximum zone of inhibition, higher than the young and mature leaves of A. cobbe. The young and mature leaves of A. cobbe and A. serratus exhibited greater zone of inhibition than the cefotaxime against the gram positive bacterium B. subtilis.

The standard antibiotic Cefotaxime exhibited 31.58 zone of inhibition against S. aureus while the aqueous and ethanolic extracts of young and mature leaves of A. serratus and A. cobbe showed the maximum zone of inhibition in the range of 20-23mm which was comparatively less than standard antibiotic. Aqueous and ethanolic extracts of young and mature leaves of A. cobbe and A. serratus exhibited good antibacterial potential against B. subtilis than S. aureus. Thus, the extracts of Allophylus leaves can be implicated to control the gram positive endospore forming bacterium B. subtilis.

2. Biocompatibility Studies

After 3 days culture, large amount of cells adhered to the surface of leaf powder. After 7 days, the population of the BMMSCs increased manifestly as compared to positive control. Favourable effects of Allophylus leaves on BMMSCs of goat which was similar to the tricalcium silicate as indicated by Jagdale et al.(2011), Bhagat et al. (2009), Singh et al. (2011) and Kumar et al.(2010) also reported compound responsible for osteogenic activity exhibiting positive results. Thus, A. cobbe and A. serratus also exhibited similar type of response in BMMSCs.