Review of literature

2.1 Isozyme polymorphism in plant populations

Genetic polymorphism in natural populations of plant and animals produced variability. Polymorphic loci revealed genetic variability while monomorphic loci are nonvariant. Isozyme are the multiple molecular forms of enzymes are common in organism. Each isozyme have specific role and exhibited tissue or cell specificity. Isozyme arise in nature by two general mechanism i.e genetic and epigenetic. The source of gene multiplicity is duplication through mutation, polyploidization and chromosomal aberrations (Hoelzel and Dover, 1991). These events constitute present epigenetic origin of isozymes. Epigenetically formed enzymes are not considered isozymes by some researcher. Epigenetic mechanisms may be divided into

a) post-translational addition,
b) post-translational deletion and
c) post-translational conformation.

There are four genetic mechanisms (Acquaah, 1992):

a) Multilocus system I – different genes code for independent proteins with same enzymatic activity. The various genes are nuclear in origin but their protein products are located in different parts of the cell.
b) Multilocus system II – is similar to system I except enzymes involved are polymeric and the subunits are encoded by more than one locus.
c) Multilocus-polymeric system – enzymes display a series of polymers that consisted of identical subunits.
d) Allozyme system – the term allozyme describe isozymes encoded by allelic genes. Alleles at various loci may be modified to produce isozymes that are distributed in a population (Wendel and Weeden, 1989).

Two measures of genetic variability was possible using electrophoresis: (1) the number of loci polymorphic (2) the average heterozygosity per an individual. Vertebrates have significantly less variation (average heterozygosity per individual) \((H = 0.060)\) than plant populations \((H = 0.121)\) (Nevo, 1978) and invertebrates may contain even higher levels of variation \((H = 0.134)\). There is also considerable variation among species within
major taxonomic groups for levels of intrapopulation variation (Powell, 1975; Nevo, 1978; Hamrick, 1979; Brown, 1979; Gottlieb, 1981).

The data on genetic variation of some major groups of plants and animals have been shown in (Table 1) as given by Ayala and Kiger (1980). The relationship between genetic variability and life history features have also been analysed within population of 113 taxa of plants. It was noted by (Hamrick et al., 1979) that life history parameters are significantly correlated several of them have significant individual effects on genetic variation. The data on genetic variability and life history traits have been shown in (Table 2).

**Taxonomic status:** The gymnosperms are more variable than either monocots or dicots. The differences in allozyme variation are probably due to the fact gymnosperms are long lived trees, are primarily outcrossed, and have high fecundities. All factors associated with high variability. Both groups contain species that represent a wide variety of longevities, mating systems, and fecundities. However, the monocots reviewed are all grasses while dicots are represented by a wider array of species (Hamrick et al., 1979).

**Geographic range and distribution:** Mean levels of genetic variation are lowest in the endemic category, increase up to the regional category, but then decrease in the widespread category. The decrease is probably due to the presence in this category of weedy, predominantly self-pollinated species (Baker, 1965).

**Generation length:** The long lived woody perennials contain the highest levels of allozyme variation. Shorter lived herbaceous perennials and annuals have intermediate levels of variation, and biennials have the least (Hamrick et al., 1979).

**Mating system:** There is a positive association between the amount of outcrossing and genetic variation that compare related species with different breeding systems (Brown, 1979; Gottlieb, 1975).

**Pollination mechanism:** Differences in levels of variability among the three categories of pollination are dramatic. Wind pollination is associated with high levels of variability (Levin and Kerster, 1974).
Table 1: Genetic variation of some major groups of plants and animals (Ayala and Kiger, 1980).

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Number of species</th>
<th>Ave number of loci per species</th>
<th>Average polymorphism</th>
<th>Average heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Invertebrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Drosophila</em></td>
<td>28</td>
<td>24</td>
<td>0.529</td>
<td>0.150</td>
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<tr>
<td>Wasps</td>
<td>6</td>
<td>15</td>
<td>0.243</td>
<td>0.062</td>
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<tr>
<td>Other insects</td>
<td>4</td>
<td>18</td>
<td>0.531</td>
<td>0.151</td>
</tr>
<tr>
<td>Marine invertebrates</td>
<td>14</td>
<td>23</td>
<td>0.439</td>
<td>0.124</td>
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<tr>
<td>Land snails</td>
<td>5</td>
<td>18</td>
<td>0.437</td>
<td>0.150</td>
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<tr>
<td><strong>Vertebrates</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fishes</td>
<td>14</td>
<td>21</td>
<td>0.306</td>
<td>0.078</td>
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<tr>
<td>Amphibians</td>
<td>11</td>
<td>22</td>
<td>0.336</td>
<td>0.082</td>
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<tr>
<td>Reptiles</td>
<td>9</td>
<td>21</td>
<td>0.231</td>
<td>0.047</td>
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<tr>
<td>Birds</td>
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<td>19</td>
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<td>Mammals</td>
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<td>28</td>
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<td>0.051</td>
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<tr>
<td><strong>Plants</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-pollinating</td>
<td>33</td>
<td>14</td>
<td>0.179</td>
<td>0.058</td>
</tr>
<tr>
<td>Outcrossing</td>
<td>36</td>
<td>11</td>
<td>0.511</td>
<td>0.185</td>
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<tr>
<td><strong>Overall averages</strong></td>
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<tr>
<td>Invertebrates</td>
<td>57</td>
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<td>0.134</td>
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<tr>
<td>Vertebrates</td>
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<td>24</td>
<td>0.247</td>
<td>0.060</td>
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<tr>
<td>Plants</td>
<td>69</td>
<td>13</td>
<td>0.345</td>
<td>0.121</td>
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Table 2: Genetic variability and life history traits (Hamrick et al., 1979).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of species</th>
<th>Mean number of loci</th>
<th>Polymorphic loci ( (P) ) (%)</th>
<th>Number alleles/locus ( (A) )</th>
<th>Polymorphic index ( (PI) )^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} )^a</td>
<td>S.E.</td>
<td>( \bar{x} )</td>
<td>S.E.</td>
<td>( \bar{x} ) S.E.</td>
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<td>Taxonomic Status</td>
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<tr>
<td>Gymnospermae</td>
<td>11</td>
<td>9.2</td>
<td>67.01</td>
<td>7.99</td>
<td>2.12 0.20</td>
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<tr>
<td>Dicotyledoneae</td>
<td>74</td>
<td>11.4</td>
<td>31.28</td>
<td>3.31</td>
<td>1.46 0.06</td>
</tr>
<tr>
<td>Monocotyledoneae</td>
<td>28</td>
<td>11.6</td>
<td>39.70</td>
<td>6.02</td>
<td>2.11 0.19</td>
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<td>Geographic Range</td>
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<tr>
<td>Endemic</td>
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<td>15.1</td>
<td>23.52</td>
<td>5.06</td>
<td>1.43 0.11</td>
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<tr>
<td>Narrow</td>
<td>22</td>
<td>11.4</td>
<td>36.73</td>
<td>6.01</td>
<td>1.60 0.14</td>
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<tr>
<td>Regional</td>
<td>39</td>
<td>8.3</td>
<td>55.96</td>
<td>5.13</td>
<td>1.85 0.10</td>
</tr>
<tr>
<td>Widespread</td>
<td>35</td>
<td>12.5</td>
<td>30.36</td>
<td>5.03</td>
<td>1.58 0.15</td>
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<tr>
<td>Generation Length</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Annual</td>
<td>42</td>
<td>11.2</td>
<td>39.47</td>
<td>4.32</td>
<td>1.72 0.11</td>
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<tr>
<td>Biennial</td>
<td>13</td>
<td>17.2</td>
<td>15.78</td>
<td>5.12</td>
<td>1.26 0.09</td>
</tr>
<tr>
<td>Short-lived Perennial</td>
<td>31</td>
<td>12.0</td>
<td>28.09</td>
<td>5.06</td>
<td>1.46 0.09</td>
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<tr>
<td>Long-lived Perennial</td>
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<td>7.6</td>
<td>65.77</td>
<td>5.08</td>
<td>2.07 0.13</td>
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<td>Mating System</td>
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<tr>
<td>Primarily Selfed</td>
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<td>14.2</td>
<td>17.92</td>
<td>3.21</td>
<td>1.27 0.06</td>
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<tr>
<td>Mixed</td>
<td>42</td>
<td>8.6</td>
<td>14.16</td>
<td>4.89</td>
<td>1.76 0.10</td>
</tr>
<tr>
<td>Primarily Outcrossed</td>
<td>36</td>
<td>11.3</td>
<td>51.07</td>
<td>4.95</td>
<td>1.85 0.12</td>
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<tr>
<td>Pollination Mechanism</td>
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<td></td>
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<tr>
<td>Selfed</td>
<td>33</td>
<td>14.2</td>
<td>18.99</td>
<td>3.51</td>
<td>1.31 0.07</td>
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<tr>
<td>Animal</td>
<td>55</td>
<td>9.5</td>
<td>38.83</td>
<td>3.94</td>
<td>1.55 0.07</td>
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<td>Wind</td>
<td>23</td>
<td>10.7</td>
<td>57.45</td>
<td>6.29</td>
<td>2.27 0.17</td>
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<td>Seed Dispersal Mechanism</td>
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<td>Large</td>
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<td>11.4</td>
<td>37.42</td>
<td>3.73</td>
<td>1.76 0.16</td>
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<td>Animal-Attached</td>
<td>16</td>
<td>11.1</td>
<td>28.79</td>
<td>5.55</td>
<td>1.55 0.08</td>
</tr>
<tr>
<td>Small</td>
<td>26</td>
<td>12.4</td>
<td>32.98</td>
<td>5.10</td>
<td>1.51 0.09</td>
</tr>
<tr>
<td>Winged or Plumose</td>
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<td>12.2</td>
<td>44.91</td>
<td>7.27</td>
<td>1.86 0.13</td>
</tr>
<tr>
<td>Animal Ingested</td>
<td>20</td>
<td>7.0</td>
<td>32.98</td>
<td>8.25</td>
<td>1.43 0.10</td>
</tr>
</tbody>
</table>

aWeighted means and standard deviations are given for each measure of variability. bDifferences in PI between categories are tested by ANOVA. Significance is indicated by * (P<0.05), ** (P>0.01), and *** (P<0.001). NS = not significant.
**Seed dispersal:** Levels of genetic variation are not strongly associated with seed dispersal distance. Seed dispersal would have much same effect as pollination: Species with restricted dispersal and therefore limited gene flow would have less genetic variation than those with greater dispersal and extensive gene flow (Hamrick et al., 1979).

Large number of plants have been investigated for the study genetic variation within population and among populations from different continents of the world.

Effect of population disjunction and isozyme variation in the widespread Pilgerodendron uviferum a conifer endemic to temperate forests of Southern America was examined. Out of the 14 genetic loci studied in twenty populations, eleven was found polymorphic. The level of isozyme variation within population was found only 11% polymorphic loci (0.95 criterion), 1.2 mean numbers of alleles per locus, and mean observed and expected heterozygosities of 0.024 and 0.033 respectively. Most of the genetic diversity was reported within population with $F_{ST}$ value of 15% (Premoli et al., 2001).

Allozyme variation have been analysed among 29 populations of Norway spruce (Picea abies (Linn.) Karst.) from Poland. Thirteen investigated isoenzyme systems were encoded by 27 gene loci and, on average, 71% of the loci per population was polymorphic. The average and effective numbers of alleles per locus was 2.17 and 1.26, respectively while expected heterozygosity was 0.156. A relatively low allozyme differentiation among populations from North Eastern and Southern Poland was observed (Lewandowski and Burczyk, 2002).

The genetic diversity in natural population of plants of heart-of-palm tree (Euterpe edulis Mart.) from Southern Brazil were analyzed using 16 allozymic loci. The results showed average 2.6 alleles per locus, with percentage of 62.5% of polymorphic loci. The mean observed heterozygosity was 0.264 and the expected heterozygosity was estimated as 0.278, greater than average of plant species studied. The genetic diversity values obtained in the population studied revealed a great capacity of the species to restore levels of diversity compatible with undisturbed populations (Conte et al., 2003).

Genetic variation in Tillandsia achyrostachys an epiphyte endemic to tropical dry forests of Mexico was examined. Comparing the genetic diversity, gene flow and genetic
differentiation in six populations of *Tillandsia achyrostachys* assessed genetic consequences of habitat fragmentation. Genetic diversity and allelic richness was: $H_e$ (expected heterozygosities) = 0.21 ± 0.02 and $A$ (mean number of alleles per locus) = 1.86 ± 0.08 respectively. F-statistics revealed a deficiency of heterozygous plants in all populations. Significant genetic differentiation between populations was detected ($F_{ST} = 0.39 ± 0.07$). Average gene flow between pairs of populations was relatively low and have high variation ($Nm = 0.46 ± 0.21$), which denotes a pattern of isolation by distance. The genetic structure of population of *Tillandsia achyrostachys* suggests habitat fragmentation have reduced allelic richness and genetic diversity, and increased significant genetic differentiation (by approx 40%) between populations (Gonzalez-Astorga et al., 2004).

Allozyme variability in three related species *Antirrhinum subbaeticum* (endangered) and *Antirrhinum pertegasii* (vulnerable) was done and then compared with *Antirrhinum pulverulentum* (non-threatened) to check the hypothesis that species with small total population size have lower levels of genetic variability than those with bigger ones. Among the three species, *Antirrhinum subbaeticum* showed mean number of alleles per locus (1.14), in *Antirrhinum pertegasii* the mean number of alleles per locus ranged between 1.15 while in *Antirrhinum pulverulentum* it ranged between 1.5 and 2.07. Total diversity ($H_T$) in *Antirrhinum subbaeticum* and *Antirrhinum pertegasii* was very low but was relatively high in *Antirrhinum pulverulentum* (Mateu-Andres, 2004).

Starch gel electrophoresis was used to describe allozyme diversity and genetic structure in 10 populations of *Macbridea alba* (Lamiaceae) an endemic Florida mint. The percentage of polymorphic loci for the species was 55% and average number of alleles per polymorphic locus was 2.91 for this species. Total genetic diversity ($H_T$) at polymorphic loci was 0.241, with most of the variation being found within populations. Nei's genetic identity values ranged from 0.93 to 0.99 indicating allele frequencies was fairly similar among populations (Godt et al., 2004).

Electrophoretic analysis of 18 allozyme loci was used to estimate levels and structuring of genetic variation within and among natural populations of the protected endemic palm species from Canary Islands (*Phoenix canariensis*) to evaluate its genetic relationship with widespread congener *Phoenix dactylifera* survey of the populations
revealed within population component explains roughly 75% of the genetic variation levels detected in *Phoenix canariensis* (*A* = 1.59; *P* = 41.8; *H*<sub>c</sub> = 0.158), which rank higher than those reported for other species of the Arecales. Reduced levels of genetic variation in *Phoenix canariensis* with respect to *Phoenix dactylifera*, the fact genetic make up of the Canarian endemic is a subset of that found in *Phoenix dactylifera* and the high genetic identity between both species strongly suggest *Phoenix canariensis* is a recently derived from a common ancestor closely related to *Phoenix dactylifera* (Gonzalez-Perez et al., 2004).

An understanding of the variation in genetic composition and population genetic structure is basic to planning the conservation of plant biodiversity. *Ballota undulata, Ballota kaiser and Ballota saxatilis* are very rare, threatened species growing in Southern Sinai, Egypt. Study of genetic diversity within and among populations of *Ballota* species demonstrated three *Ballota* species maintain relatively high levels of genetic diversity (*H*<sub>c</sub> = 0.195-0.317) and most of their genetic diversity was found within populations (*G*<sub>ST</sub> = 0.045-0.099). Hence the conservation of genetic diversity naturally occurring in these species should still be possible by a combination of in situ and ex situ conservation efforts (Zaghloul et al., 2006).

The allozyme markers was used to assess the genetic diversity in the four population of *Terminalia paniculata* Roth. in order to suggest conservation and management strategies from Western Ghat, India. Six enzyme systems generated 15 loci from four populations which was used to estimate allele frequency, percentage of polymorphic loci, observed heterozygosity (*H*<sub>o</sub>) and expected heterozygosity (*H*<sub>e</sub>) and Shannon information index. In the Sasthakovil populations 12 rare alleles, two private alleles (AAT-1C and PGM-2C), and a high level of polymorphism 86.66% was observed. The levels of heterozygosity observed in all populations was lower than expected from Hardy-Weinberg equilibrium values, except Sasthakovil populations. As a priority, the population at Sasthakovil may be targeted for conservation. This would ensure the conservation of a relatively rich proportion of genetic diversity and presence of private allele representative of that existing in other populations (Thangaraja and Ganesan, 2007).
**Vitex rotundifolia** (Linn.) is an important plant species used in traditional Chinese medicine. For its efficient use and conservation, genetic diversity and clonal variation of *Vitex rotundifolia* populations in China was investigated using inter-simple sequence repeat markers. Fourteen natural populations were included to estimate genetic diversity, and a large population with 135 individuals was used to analyze clonal variation and fine scale spatial genetic structure. The overall genetic diversity (GD) of *Vitex rotundifolia* populations in China was moderate (GD = 0.190), with about 40% within population variation across all populations surveyed, the average within population diversity was moderate (P = 22.6%; GD = 0.086). A relatively high genetic differentiation (GST = 0.587) among populations was detected based on the analysis of molecular variance data (Hu et al., 2008).

The allozyme variability were screened at 19 loci for a total of 858 individuals from 27 populations of *Narcissus longispathus*, were collected from South Eastern Spanish mountains using horizontal starch gel electrophoresis. *Narcissus longispathus* displayed high levels of genetic diversity and extensive diversification among populations. At the species level, the percentage of polymorphic loci was 68%, with average values of 2.1, 0.11 and 0.14 for the number of alleles per locus, observed heterozygosity and expected heterozygosity, respectively. Southern and more isolated populations tended to have less genetic variability than Northern and less isolated populations. A strong spatial patterning of genetic diversity was found at the various spatial scales. Gene flow/drift equilibrium occurred over distances < 4km. Beyond that distance divergence was relatively more influenced by drift. The populations studied seem to derive from three panmictic units or gene pools, with levels of admixture being greatest in the Central and South Eastern portions of the species range (Medrano and Herrera, 2008).

Allozyme diversity of 31 populations along its distribution range via starch gel electrophoresis, have been studied in *Delphinium staphisagria* from Mediterranean Basin by assaying 12 enzyme systems and scoring 17 loci. The low levels of genetic variability have been detected (A = 11.8, A_p = 1.6, H_o = 0.026, H_e = 0.057) (Orellana et al., 2009).

Genetic diversity was measured by isozymes in 25 accessions of the medicinal climber *Tinospora cordifolia* (Wild.) from Gujarat, India. Analysis of ten isozymes
revealed presence of 16 gene loci and 33 alleles in 25 accessions. The percentage of polymorphic loci (P) was 45.0% and mean observed number of alleles per locus (A) was 1.57. The average observed heterozygosity (H_o) and expected heterozygosity (H_e) was 0.443 and 0.270, respectively show high levels of genetic variation among different accessions (Kalpesh and Mohan, 2009).

The genetic diversity and relationship between populations in different habitats have been studied in Glycyrrhiza uralensis by amplified fragment length polymorphism (AFLP) of North China. The plant materials consisted of 50 individuals from 5 different populating areas of Chifeng (Inner Mongolia), Hengjinq (Inner Mongolia), Minqin (Ganshu), Aletai (Xinjiang) and Kashi (Xinjiang). The genetic resources and diversity in wild populations of Glycyrrhiza uralensis was rich (polymorphism = 54.3%, H_e = 0.1932). The polymorphism among populations was Chifeng > Hengjinq > Minqin > Aletai > Kashi and the genetic diversity varied from 0.1794-0.2061 and was in the order of Minqin > Chifeng > Hengjinq > Aletai > Kashi (Zhang et al., 2010).

The isozyme electrophoresis was used to study the genetic variation within and among 22 populations of the Carex magellanica subspecies irrigua from Estonia, Fennoscandia and South Central Alaska of the nine putative isozyme loci assessed, five 56% was found to be polymorphic. The genetic diversity in small and fragmented Estonian populations was lower (H_e = 0.034) than in larger Fennoscandian and Alaskan populations (average H_e = 0.082). All standard genetic parameters (A_e, H_o, H_e, P, F_ST) showed lowest values in Estonian populations. The heterozygosity level in Fennoscandian populations was low (H_e = 0.01), whereas no heterozygotes was found in Estonian and Alaskan populations. High F_ST values indicate Carex magellanica subspecies irrigua is predominantly inbreeding (Kull and Oja, 2010).

Knowledge of genetic structure and variability is critical for the formulation of scientifically based, species-specific conservation and restoration. Hence the present study was planned to access the genetic variability in Achyranthes aspera for the development of efficient conservation strategies.

2.2 Antibacterial activity of medicinal plants

The use of medicinal plants all over the world predates the introduction of antibiotics in different parts of the world. Herbal medicine have been widely used and
formed integral part of primary health care in India, China, South Africa, Ethiopia, Argentina and Papau New Guinea etc. In recent times, the rapid development of multiresistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents. The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antibacterial activity. A number of herbs with significant antibacterial activity have been reported in different traditional literatures (Satish et al., 1999).

The antimicrobial activity of crude ethanolic extracts of 10 medicinal plants raised from seeds obtained from France and Japan used in traditional Chinese medicine were tested against five species of microorganisms. *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Of the 10 plants tested, 5 showed antimicrobial activity against one or more species of microorganisms. The most active antimicrobial plants were *Chelidonium majus*, *Sanguisorba officinalis*, and *Tussilago farfara* (Janovska et al., 2003).

*Achyranthes bidentata* Blume. (Amaranthaceae) was investigated for antibacterial activity against *Bacillus subtilis* (NCIM 2439), *Staphylococcus aureus* (NCIM 2492), *Pseudomonas aeruginosa* (NCIM 2053) and *Escherichia coli* (NCIM 2068) organisms, using agar diffusion method. The petroleum ether, chloroform, methanol and aqueous extracts showed significant antibacterial activity. Our findings offer experimental support to the therapeutic claims on this herb as useful against bacterial infections (Balakrishnan et al., 2003).

3-Acetoxy-6-benzoyloxyapangamide have been isolated from an ethylacetate extract of the stem of *Achyranthes aspera*. The structure of the isolated compound was established by modern spectroscopic techniques. The extract was found to show mild antibacterial activity against *Bacillus cereus* (Aziz et al., 2005).

*Achyranthes aspera* (Linn.) extracts was evaluated for antibacterial activity against human pathogenic bacterial strains of Gram positive *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Bacillus subtilis* and Gram negative *Escherichia coli*. The methanolic extract showed maximum activity against *Streptococcus salivarius* and petroleum ether
extract showed minimum activity against *Escherichia coli*. The use of extracts of *Achyranthes aspera* as a potential antibacterial activity and treatment of dental plaque have been suggested (Prabhat et al., 2005).

Six Nigerian medicinal plants *Terminalia avicennioides, Phylantus discoideus, Bridella ferruginea, Ageratum conyzoides, Ocimum gratissimum* and *Acalypha wilkesiana* used by traditional medical practitioners for the treatment of several ailments of microbial and non-microbial origins was investigated for *in vitro* anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity. Both water and ethanol extracts of *Terminalia avicennioides, Phylantus discoideus, Ocimum gratissimum, and Acalypha wilkesiana* were effective on MRSA. The Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanol extracts of these plants ranged from 18.2 to 24.0mcg/ml and 30.4 to 37.0mcg/ml respectively. In contrast, MIC ranged of 30.6 to 43.0mcg/ml and 55.4 to 71.0mcg/ml were recorded for ethanol and water extracts of *Bridella ferruginea* and *Ageratum conyzoides* respectively. Higher MBC values were obtained for the two plants. All the six plants exhibited presence of alkaloids, tannins and saponins, however anthraquinone was found in the four active plants (Akinyemi et al., 2005).

Antimicrobial effect of the crude organic extract of *Xylocarpus granatum* stem barks was studied in the Department of Pharmacology, Bangladesh with disc diffusion method the extract showed significant antimicrobial activity against *Staphylococcus epidermis, Staphylococcus aureus, Shigella boydii, and Proteus spp.* and moderate activity against *Escherichia coli, Streptococcus pyogenes*, and no activity against *Shigella dysenterii, Enterococci, Salmonella typhi* (Alam et al., 2006).

Eighteen plant species used in folklore medicine in West Nepal were tested for their antibacterial activity by the disc diffusion method. The bacteria employed were gram positive *Staphylococcus aureus* and gram negative *Escherichia coli, Pseudomonas aeruginosa* and *Shigella boydii*. Extracts of eight plants showed encouraging result against three strains of bacteria while other showed activity against one or two strains (Panthi and Chaudhary, 2006).

*In vitro* antimicrobial activities of the aqueous extraction of neem (*Azadirachta indica*) leaves, seeds and seed hulls along with their sulphates was carried out. All of
these native extracts and their corresponding sulfates at concentration of 20µg/disc gave various inhibition activities against Grampositive bacteria Staphylococcus aureus, the Gram negative bacteria Escherichia coli, the yeasts Candida albicans, yeast-like fungus, the fungi Aspergillus niger and Penicillium citrinum. Generally, the high level of protein and carbohydrate contents of extract have better fibrinolytic and antimicrobial activities (Helmy et al., 2007).

Antimicrobial activity of methanol extract of Diospyros peregrina fruits (MEDP), Coccinia grandis leaves (MECG) and Swietenia macrophylla barks (MESM) was examined against some selective gram positive and gram negative bacterial (20) and fungal (4) strains. Diospyros peregrina fruits and Swietenia macrophylla barks both have been shown highest sensitivity against Escherichia coli strains. Diospyros peregrina fruits was found resistant to Sarcina luteus and Bacillus spp. whereas Swietenia macrophylla barks was resistant to all Shigella strains. Coccinia grandis leaves have been shown major activity against Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Shigella sonnei and Pseudomonas aeruginosa; whilst resistant to Shigella flexneri and Shigella boydii. Against fungi strains extracts was found effective at higher concentrations. Candida albicans have been shown highest sensitivity whilst Penicillium spp. was least effective to all three extracts (Dewanjee et al., 2007).

In vitro antimicrobial activity of methanolic extracts of some medicinal plants was evaluated against Escherichia coli, Salmonella typhimurium, Staphylococcus aureus and Enterococcus sp. The methanolic extract of Caryophyllus aromaticus presented highest anti-Staphylococcus aureus activity and were effective against all bacterial strains tested (Ushimaru et al., 2007).

Antimicrobial properties of leaf extracts of Senna obtusifolia were collected from Nigeria against both clinical and laboratory isolates of four gram negative bacteria; Neisseria gonorrhoeae, Salmonella sp., Pseudomonas aeruginosa, Proteus vulgaris and two gram positive bacteria; Staphylococcus aureus and Streptococcus aerugensosa using disc diffusion method. Acetone extracts (12mm zone diameter of inhibition, MIC 200µg/ml and MBC 300µg/ml) demonstrated highest activity followed by dichloromethane (8mm zone diameter of inhibition, MIC 300µg/ml and MBC 400µg/ml). Methane (7mm zone diameter of inhibition, MIC 400µg/ml and MBC 400µg/ml) and hexane (6mm zone
diameter of inhibition, MIC 800μg/ml and MBC 1000μg/ml). Water extracts demonstrated least activity against test bacteria and fungi (4mm zone diameter of inhibition, MIC 800μg/ml and MBC 800μg/ml). Pytoconsitituents present included saponins, tannins, alkaloids and flavonoids. *Senna obtusifolia* can be to source antibiotic substances for possible treatment of bacterial and fungal infection including gonorrhea, pneumonia, urinary tract and some mycotic infections (Doughari et al., 2008).

Methanolic extracts obtained from different parts (leaves, flowers, stem and pods) of *Cassia surattensis* and *Cassia fistula* were collected from Malaysia was evaluated for potential antimicrobial activity against medically important bacterial, yeast and fungal strains. Methanol extracts of *Cassia surattensis* and *Cassia fistula* were effective on tested microorganisms. The methanolic extract with greatest antimicrobial activity was from stem of *Cassia surattensis* and *Cassia fistula*. *Micrococcus sp.*, *Salmonella typhi*, *Escherichia coli*, *Azospirillum lipoferum*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans* were most susceptible strains while most resistant strain was *Saccharomyces cerevisiae* (Sangetha et al., 2008).

Extracts in organic solvents (namely methanol, ethanol, ethylacetate and chloroform) of two medicinal plants – *Achyranthes aspera* and *Cassia alata* was evaluated for their antibacterial activities against *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi* and *Staphylococcus aureus*. The leaf and stem parts of *Achyranthes aspera* did not reveal antibacterial activity in any organic extractions. The methanolic extracts of both leaf and stem parts of *Cassia alata* exhibited antibacterial activity, but only to *Bacillus subtilis* and *Salmonella typhi*, and the corresponding MIC values of the leaf extracts was estimated as 1.25 and 1.5mg/ml respectively. However, the ethanolic extracts of both stem and leaf parts was found equally effective against *Staphylococcus aureus* (MIC = 1.25mg/ml) (Alam et al., 2009).

*In vitro* antibacterial activity of aqueous and methanol extracts of some medicinal plants *Acacia holosericea*, *Ipomoea carnea*, *Justicia gendurussa* and *Withania somnifera* were screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* and *Streptococcus pyogenes* isolated from clinical specimen. Methanol extracts of
*Withania somnifera* showed higher antibacterial activity compared to other plant extracts tested. The minimum inhibitory concentration of methanol extracts of *Withania somnifera* was in the range 50 to 100mg/ml. Phytochemical studies on the leaf extracts of medicinal plants revealed presence of alkaloids, saponins, cardiac glycosides, steroids, tannins and flavonoids (Rajendran and Ramakrishnan, 2009).

Methanol, hexane and aqueous extracts of *Callistemon viminalis* were tested against eight common bacteria and a single fungus of medical importance using a quantitative agar well diffusion test and tube dilution assay. All plant extracts showed antimicrobial activities against selected microorganisms; the methanol extracts were most effective. The aqueous extract on the other hand was very effective as a bactericidal agent against intestinal pathogens. These results support ethanomedicinal claim *Callistemon viminalis* is an effective treatment for bacterial caused of intestinal illnesses (Delahaye et al., 2009).

Antimicrobial activities of the crude ethanolic extracts of five plants were screened against multidrug resistant (MDR) strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*. ATCC strains of *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus bovis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* were also tested. The (MDR) strains were sensitive to the antimicrobial activity of *Acacia nilotica*, *Syzygium aromaticum* and *Cinnamomum zeylanicum* where as they exhibited strong resistance to the extracts of *Terminalia arjuna* and *Eucalyptus globulus*. Community acquired infections showed higher sensitivity than nosocomial infections against these extracts. The most potent antimicrobial plant was *Acacia nilotica* (MIC ranged 9.75-313µg/ml), where as other crude plant extracts studied in this report was found to exhibited higher MIC values than *Acacia nilotica* against community acquired as well as nosocomial infection (Khan et al., 2009).

The extract of *Cinnamomum tamala* was evaluated for antibacterial potential by agar well diffusion assay against a total of six bacterial strains. Almost of all extracts evaluated showed variable degree of inhibition zones against different bacterial species except hexane extract which was found completely inactive. Other organic extracts ethanol, methanol, ethylacetate was found significant activity against all test bacteria
except *Escherichia coli* which was observed completely resistant to all the extracts. Methanol extract was found comparatively more effective (Goyal et al., 2009).

The antibacterial properties of selected medicinal plant *Viz Ocimum sanctum* (Tulsi), *Origanum majorana* (Ram tulsi), *Cinnamomum zeylanicum* (Dalchini) and *Xanthoxylum armatum* (Timur) of Nepal were screened against 10 medically important bacterial strains, *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Staphylococcus aureus, Pseudomonas spp., Proteus spp., Salmonella typhi, Escherichia coli, Shigella dysenteriae, Klebsiella pneumoniae*. The most susceptible bacteria was *Bacillus subtilis* followed by *Staphylococcus aureus* while most resistant bacteria was *Escherichia coli* followed by *Shigella dysenteriae, Klebsiella pneumoniae* and *Salmonella typhi*. *Origanum majorana* showed best antibacterial activity. The largest zone of inhibition were obtained with *Xanthoxylum armatum* against *Bacillus subtilis* (23mm) (Joshi et al., 2009).

The antibacterial activity of various extracts of whole plant of *Borreria hispida* (Linn.) was evaluated against different bacteria. All the extracts of *Borreria hispida* were tested for antibacterial efficacy against *Bacillus subtilis* (NCIM 2063), *Bacillus pumilus* (NCIM 2327), *Staphylococcus aureus* (NCIM 2079), *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2065), *Klebsiella pneumoniae* (NCIM 2957). All the three extracts was comparable with standard drug (ciprofloxacin). The methanolic extract was found to be the most effective antibacterial activity against entire organism tested. The minimum inhibitory concentration of methanolic extract of *Borreria hispida* was found in the ranged of 250mcg/ml to 50mg/ml on tested all the test organisms. This study scientifically supports usage of whole plant as a remedy for various superficial bacterial and fungal infections in traditional medicine (Muthu et al., 2010).

*Cassia occidentalis, Croton zambesicus* and *Newbouldia* leaves are known as ‘Ewe ori esi’, Ajekobale and Akoko respectively in Yoruba are three plants whose leaves are used in combination by boiling to treat gastrointestinal and diarrhea illnesses among indigenes of Akungba-Akoko, Ondo State, Nigeria. The leave extracts from these medicinal plants were screened *in vitro* in the laboratory for their antibacterial activity against two prominent enteric bacteria, *Escherichia coli* and *Salmonella typhimurium*. The tyndalized leave extract of *Croton zambesicus* showing antibacterial inhibition zone
of 4 and 2mm against *Salmonella typhimurium* and *Escherichia coli* respectively exhibited highest activity than autoclaved samples and other plant sources tested independently or combined, showing combinations of the extract samples did not exhibit synergistic effects. *Croton zambesicus* can however be optimized clinically for chemotherapeutic control of these food borne enteric infections (Ajayi and Akintola, 2010).

The antibiotic activity of aqueous and ethanol extracts of the root bark of two plants; *Alstonia boonei* (De wild.) and *Morinda lucida* locally used in Ibadan for treating different diseases especially fever was studied against seven bacteria. Crude extracts obtained from *Alstonia boonei* were not potent against any of the bacterial tested. *Bacillus subtilis* were most sensitive organism to the ethanol root bark extracts of *Morinda lucida* while *Pseudomonas aeruginosa* was least sensitive. Difference in antibacterial activity of these plants showed different bioactive components are present in each species. Preliminary phytochemical test of *Morinda lucida* plant parts showed presence of saponins, anthraquinone, cardenolides, alkaloids but absence of tannins (Adomi and Umukoro, 2010).

The aqueous, methanol and acetone extracts of *Garcinia kola* seeds was evaluated for their antibacterial activity against four *Staphylococcus* strains isolated from wound sepsis specimens. The aqueous, methanol and acetone extracts of *Garcinia kola* seeds showed activity against all four isolates at 30mg/ml (aqueous extract) and 10mg/ml (acetone and methanol extracts). The MIC values for the aqueous extract were same 10mg/ml for all the isolates. The acetone and methanol extracts have lower MIC values in the ranged of 0.3125-0.625mg/ml (Sibanda et al., 2010).

### 2.3 Anti-inflammatory activity of medicinal plants

Inflammation (Latin, inflammare, to set on fire) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Werts et al., 2007). Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. Inflammation can be classified as either acute and chronic. Acute inflammation having a
rapid onset and coming to a crisis relatively quickly, with clear and distinct termination. Chronic inflammation that may have rapid or slow onset but is characterized primarily by its persistence and lack of clear resolution; it occurs when tissues are unable to overcome effects of the injuring agent (Cotran et al., 1999). Medicinal plants play the significant role in the treatment of inflammation.

Effect of *Azadirachta indica* leaf extract on inflammatory oedema induced by chemical mediators (5-HT, histamine, bradykinin) was studied to find out its possible mechanism of reported anti-inflammatory effect against carrageenan induced rat hind paw oedema. The test material showed significant anti-inflammatory effect against 5-HT induced inflammation but not on the inflammation induced by histamine and bradykinin. Median effective doses of *Azadirachta indica* for 5-HT induced oedema were 1.17g and 930mg/kg respectively. The result suggests *Azadirachta indica* extract’s anti-inflammatory effect may be due to antagonism of the deleterious effect of 5-HT on blood vessels (Chattopadhyay et al., 1993).

*Saussurea lappa, Argyreia speciosa* and *Achyranthes aspera* are well known Indian medicinal plants used in the indigenous systems of medicine for the treatment of inflammatory conditions. The ethanolic extracts of the plants at the doses of (50, 100 and 200mg/kg p.o.) were screened for their effect on acute and chronic inflammation induced in mice and rats. *Saussurea lappa* and *Argyreia speciosa* was found to significantly inhibit paw edema induced by carrageenan and freund's complete adjuvant and to prevent accumulation of inflammatory cells in carrageenan induced peritonitis at doses of 50-200mg/kg. *Achyranthes aspera* inhibited these inflammatory responses at doses of 100-200mg/kg (Gokhale et al., 2002).

The anti-inflammatory activity of alcoholic extract of *Achyranthes bidentata* on carrageenan induced hind paw oedema and cotton pellet granuloma models in swiss male rats. The hind paw oedema was produced by subplantar injection of carrageenan and the paw volume was measured plethysmographically at 0, 1, 2, 3, 4 and 5h. In sub-acute model, cotton pellet granuloma was produced by implantation of 50 ±1mg sterile cotton in axilla under ether anaesthesia. The animals was fed with ethanolic extract at various dose levels 125, 250, 375 and 500mg/kg. Diclofenac sodium was used as a standard drug. The alcoholic extract 375 and 500mg/kg showed maximum inhibition of oedema by
63.52% and 79.73% at the end of 3h in acute model of inflammation, respectively. Using a chronic test, the granuloma pouch in rats, the extract exhibited a 50.76% and 57.49% reduction in granuloma weight (Vetrichelvan and Jagadeesan, 2002).

The acute and chronic anti-inflammatory activity of leafy exudate of Aloe vera (AVL) in animal models of inflammation. The acute anti-inflammatory activity of Aloe vera was evaluated using carrageenan and dextran as phlogistic agents while its chronic anti-inflammatory effect was investigated in a complete freund's adjuvant induced model of arthritis. The degree of inflammation in all models was measured plethysmographically. The effect of Aloe vera on nitric oxide production in mouse peritoneal macrophages was measured by the Griess reagent. Aloe vera 25mg/kg significantly reduced carrageenan and dextran induced pedal edema in rats by 61.9% and 61.7%, respectively. In the freund's adjuvant induced model of chronic inflammation, Aloe vera showed chronic anti-inflammatory activity but failed to decrease arthritic index indicating absence of antiarthritic activity. Aloe vera 10µg/ml caused decrease in nitric oxide production in macrophages without causing toxicity (Sarkar et al., 2005).

Curcumin, a natural compound present in the rhizomes of plant Curcuma longa (Linn.) was studied in India for its anti-inflammatory action. Curcumin was found to inhibit arachidonic acid metabolism, cyclooxygenase, lipoxygenase, cytokines (Interleukins and tumour necrosis factor) Nuclear factor-kB and release of steroidal hormones. Curcumin was reported to stabilize lysosomal membrane and caused uncoupling of oxidative phosphorylation besides having strong oxygen radical scavenging activity, which was responsible for its anti-inflammatory property. In various animal studies, a dose ranged of (100-200mg/kg b.wt.) exhibited good anti-inflammatory activity and seemed to have negligible adverse effect on human systems. Oral (LD<sub>50</sub>) in mice was found to be more than (2.0gm/kg b.wt.) (Kohli et al., 2005).

Another study from India a two polyherbal Ayurvedic formulations Chandraprabha vati (CPV) and Maha yogaraja guggulu (MYH) reported anti-inflammatory activity of the formulations, in carrageenan induced rat paw edema model was used. Chandraprabha vati and Maha yogaraja guggulu showed dose dependent anti-inflammatory activity with maximum 45% and 49% in paw edema respectively at a dose of 500mg/kg (Bagul et al., 2005).
A study from Cameroon on the leaf extracts of *Kalanchoe crenata* obtained in methylene chloride/methanol extract and further extracted successively with hexane, methylene chloride, ethylacetate, and n-butanol was conducted in paw edema induced by carrageenan. The n-butanol fraction (most potent) was further assessed through acute inflammatory models induced by histamine, serotonin, and formalin. The chronic anti-inflammatory and the ulcerogenic activities of the n-butanol fraction was also examined. The oral administration of n-butanol fraction 600mg/kg caused maximum inhibition of about 45% in paw edema induced by carrageenan. The n-butanol fraction also exhibited acute anti-inflammatory activity on paw edema induced by histamine 47.51%, serotonin 54.71%, and formalin 40.00%. In the chronic inflammation model, this extract showed maximum inhibition of 61.26% on the ninth day of treatment. The ulcerogenic assessment showed ulcer indices after oral treatment with n-butanol fraction were zero and 0.4 ± 0.2, for the 300 and 600mg/kg doses, respectively (Dimo et al., 2006).

Hexanic, dichloromethanic, ethanolic and aqueous extracts from *Baccharis obtusifolia*, *Baccharis latifolia*, *Baccharis pentlandii* and *Baccharis subulata* plants used in the traditional medicine of South America have been studied for their in vitro anti-inflammatory activity in cellular systems. Calcium ionophore A23187-stimulated mouse peritoneal macrophages were validated as a source of cyclooxygenase-1 (COX-1) (prostaglandin E\textsubscript{2}, PGE\textsubscript{2}) and 5-lipoxygenase (5-LOX) (leukotriene C\textsubscript{4}, LTC\textsubscript{4}), and mouse peritoneal macrophages stimulated with *Escherichia coli* lipopolysaccharide (LPS) was used for testing cyclooxygenase-2 (COX-2) (PGE\textsubscript{2}), nitric oxide (NO) and tumour necrosis factor-α (TNF-α) activity. Most of the extracts tested were active in all assays (Abad et al., 2006).

The methanolic extract of dried leaf callus of *Silybum marianum* (Linn.) Gaertn. was examined for anti-inflammatory activity, using carrageenan and formalin induced rat paw oedema models, which was also compared with leaf extract. Leaves were collected from the plants grown in the herbal garden of Hamdard University, New Delhi. The leaf and leaf callus of *Silybum marianum* inhibited formation of paw oedema to significant levels in rats treated either with carrageenan or formalin. At a dose of 100mg/kg orally, the leaf extract produced 74% inhibition while leaf callus produced 93.9% inhibition in case of the carrageenan induced oedema (P<0.01) and there was 85.61% inhibition in leaf extract and 91.27% inhibition in leaf callus extract, in formalin induced oedema (P<0.01). The %
inhibition showed by leaf callus extracts 100mg/kg was found to be more than reference standard i.e., aspirin 93.9% vs 78.79% inhibition in carrageenan induced rat paw oedema, and 91.27% vs 86.86% inhibition in formalin induced rat paw oedema (Balian et al., 2006).

The extract obtained from the *Thalassia testudinum* demonstrated an inhibitory effect in the carrageenan paw oedema. Chloroform and butanolic fractions also inhibited mouse ear oedema assay. The extract, butanolic and aqueous fractions inhibited writhing test. We can conclude extract, chloroform and butanol fractions showed anti-inflammatory properties (Llanio et al., 2006).

The methanolic extracts of *Hippocrates indica* root bark and *Poga oleosa* fruits were collected from Abeokuta, Ogun State for anti-inflammatory activity. Both extracts inhibited carrageenan induced paw oedema significantly in rats in a dose dependent manner in 4h. *Hippocrates indica* inhibited oedema significantly at the minimal dose 125mg/ml (p<0.05) from 2h onward, and gave 100% inhibition in 4h at 250mg/kg. It have been shown to be a more potent anti-inflammatory agent than *Poga oleosa*. These results confirm anti-inflammatory activities of the two plants (Ogbole et al., 2007).

The effects of methanol extract of *Phyllanthus amarus* were collected Belgaum district Karnataka, India on different phases of inflammation was examined. Investigations were performed using different phlogistic agents induced paw edema, carrageenan induced air pouch inflammation and cotton pellet granuloma in rats. Methanol extract of *Phyllanthus amarus* significantly inhibited carrageenan, bradykinin, serotonin and prostaglandin E1 induced paw edema, but failed to inhibit histamine induced paw edema. Maximum inhibition was observed in prostaglandin E1 induced paw edema. In carrageenan air pouch model, methanol extract of *Phyllanthus amarus* significantly reduced volume of exudate and migration of neutrophils and monocytes. The extract significantly decreased formation of granuloma tissue in chronic inflammation model. The methanol extract of *Phyllanthus amarus* inhibits all the phases of inflammation (Mahat and Patil, 2007).

50% ethanolic extract of the aerial part of *Cissampelos pareira* (Linn.) var. Hirsuta (Menispermaceae) were collected from National Botanical Research Institute, India tested for anti-inflammatory (paw edema induced by carrageenan and arachidonic acid) and analgesic activity (abdominal writhes and hot plate) in rats and mice, respectively. Oral administration of extract exhibited significant and dose dependent anti-inflammatory activity in the
carrageenan test, which was based on interference with prostaglandin synthesis, as confirmed by the arachidonic acid test. In the abdominal writhing test induced by acetic acid, higher dose of the plant extract have the highest analgesic activity, whereas in the hot plate test the best dose was 100mg/kg (P<0.05). The (LD$_{50}$) showed *Cissampelos pareira* 2000mg/kg presented low toxicity (Amresh et al., 2007).

Essential oil of Bergamot (BO) were collected from Antalya, Turkey was evaluated for anti-inflammatory activity using carrageenan induced rat paw oedema test. It was found that reduction in inflammation was 95.70% with indomethacin a reference agent, 27.56% with 0.025ml/kg BO, 30.77% with 0.05ml/kg BO and 63.39% with 0.10ml/kg BO. Indomethacin showed strongest anti-inflammatory activity among the drugs used. The strongest anti-inflammatory activity of Bergamot oil was seen with 0.10ml/kg dosage. Median effective dose (ED$_{50}$) value of Bergamot oil was found to be 0.079ml/kg (Karaca et al., 2007).

Aqueous, ethanolic and methanolic extracts of *Ruta graveolens* were collected from Kannur district, Kerala, India was investigated for anti-inflammatory activity in carrageenan induced paw edema in wistar male rats and compared to a positive control drug voveran. Methanolic extracts of *Ruta graveolens* with concentration of (20mg/kg b.wt.) and ethanolic extract with concentration of (50mg/kg b.wt.) showed maximum 90.9% inhibition on carrageenan induced rat paw edema. The effect were significantly (P<0.05) higher than standard drug voveran 72.72%. Methanol extract with concentration of (50mg/kg b.wt.) produced 81.81% inhibition, which was also high as compared to the standard drug. Ethanolic extract with dose of (20mg/kg b.wt.) and the two doses of aqueous extract produce less percentage of inhibition as compared to the standard drug voveran (Ratheeesh and Helen, 2007).

Thirty-seven species of common seaweeds from the coast of Korea were screened for anti-inflammatory activity. Methanol extracts of the seaweeds were tested against mouse ear edema and erythema induced by phorbol myristate acetate. At 40mg/ml of extract, edema was strongly suppressed by the seaweeds *Undaria pinnatifida* and *Ulva linza*, with relative inhibition of 85% and 84%, respectively (Khan et al., 2008).

The methanol leaves extract of *Cissampelos mucronata* (Linn.) A. Rich from Nigeria was investigated for possible antinociceptive (analgesic) and anti-inflammatory
effects in mice and rats. The antinociceptive study was carried out using the acetic acid induced abdominal constriction test in mice and tail immersion test in rat while anti-inflammatory effect was investigated by employing the carrageenan induced paw oedema in rats. The extract significantly (P<0.001) inhibited carrageenan induced paw inflammation at the third hour. Preliminary phytochemical screening of the extract revealed presence of carbohydrates, reducing sugars, combined sugars, flavonoids, tannins, saponins, glycosides, alkaloids and steroids. The intraperitoneal (LD_{50}) of the extract, found to be (3807.9mg/kg b.wt.) in mice, suggests extract is relatively non toxic at the analgesic and anti-inflammatory dose (Yaro et al., 2008).

The flavonoid fraction from alcoholic extract of the leaves of *Celosia argentea* (Linn.) (Amaranthaceae) were collected from Pune and Sinhgad was investigated for anti-inflammatory activity in animal models. Results revealed flavonoid possesses significant anti-inflammatory properties when investigated by employing carrageenan induced rat paw edema and cotton pellet induced chronic inflammatory models. The significant dose dependent anti-inflammatory activities in both models and also confirmed flavonoids of *Celosia argentea* are responsible for anti-inflammatory activity (Bhujbal et al., 2008).

The aqueous extract of the stem bark of *Cussonia paniculata* were collected from South Africa was investigated for its anti-inflammatory and analgesic activities in animal models. The extract at (50, 100 and 200mg/kg b.wt.) reduced significantly, the formation of oedema induced by carrageenan and histamine. In the acetic acid induced writhing model, the extract showed good analgesic effect characterized by reduction in number of writhes when compared to the control. The extract caused dose dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin, signifying its analgesic effect. These results were also comparable to those of indomethacin and cyproheptadine, the reference drugs. Acute toxicity test showed plant caused 80% mortality in rats, hence it is a toxic plant (Adedapo et al., 2008).

*Bauhinia purpurea* (Linn.) (Leguminosae), choloform extract of leaves from Malaysia was evaluated for antinociceptive and anti-inflammatory activities of using animal models. The results obtained indicate extract possess significant (p<0.05), but concentration independent, antinociceptive activity when assessed using the abdominal constriction and hot plate test. This activity was also, significantly (p<0.05) observed in the early and late
phases of the formalin test. The extract also exhibited significant (p<0.05) anti-inflammatory activity in a non concentration dependent manner. Unexpectedly, the 100mg/kg extract showed less remarkable anti-inflammatory activity compared to the other doses tested (Zakaria et al., 2009).

The anti-inflammatory activities of the methanol extract of *Dillenia indica* (Linn.) (Dilleniaceae) leaves were collected from Mumbai, India observed in various experimental models related to inflammation to provide some evidence for its traditional use. Anti-inflammatory activity was observed in carrageenan induced edema and acetic acid induced capillary permeability. The methanol extract showed significant (P<0.01) anti-inflammatory activity in the paw edema test and acetic acid induced capillary permeability at 200mg/kg and 400mg/kg. The extract at 100mg/kg showed significant (P<0.05) activity in acid induced permeability (Yeshwante et al., 2009).

The methanolic extract of leaves of *Eclipta prostrata* (Linn.) was investigated for anti-inflammatory activity in albino wistar rats were collected from Tamilnadu, India. The methanolic extract administered by the oral route at a concentration of 100 and 200mg/kg showed significant dose dependent anti-inflammatory activity in carrageenan and egg white induced hind paw oedema in rats. Anti-inflammatory activity of the tested extract was comparable with standard drug indomethacin 10mg/kg and cyproheptadine 8mg/kg (Arunachalam et al., 2009).

The extract of the bark of *Albizia lebbeck* Benth. obtained by cold extraction of mixture of equal proportions of petroleum ether, ethyl acetate and methanol was chosen for pharmacological screening. In rat paw edema model induced by carrageenan, the extract at the 400mg/kg dose level showed 36.68% (p<0.001) inhibition of edema volume at the end of 4h. In the acetic acid induced writhing test, the extract at the 200 and 400mg/kg dose level showed 39.9% and 52.4% inhibition of writhing (Saha and Ahmed, 2009).

*Borassus flabellifer* (Linn.) (Areceae) were collected from Uttar Kannada district Karnataka widely used for its reported biological activities in indigenous system of medicine. To find the effect of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* for its anti-inflammatory activity in rodents. The anti-inflammatory activity was evaluated using acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton pellet induced granuloma and carrageenan induced
air pouch model in rats. Oral administration of the extract at the doses (150 and 300mg/kg b.wt.) exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan induced hind paw oedema, (p<0.0001) and chronic (cotton pellet granuloma and carrageenan induced air pouch models, (p<0.0001) of inflammation. The extract also showed significant (p<0.0001) results for biochemical parameters (Paschapur et al., 2009).

The methanolic extract of Acalypha indica (Linn.) also demonstrated anti-inflammatory effect in a dose dependent manner in mice. Maximum inhibition by the extract was observed at (250mg/kg b.wt.) after three hours of ingestion, which was comparable to the standard drug phenylbutazone at a dose of (100mg/kg b.wt.) (Rahman et al., 2010).

Inflammation is a response of vascularized living tissue to the local injury. The severe side effects of steroidal and non-steroidal anti-inflammatory drugs evoked us to search for new anti-inflammatory drugs from the indigenous source. The methanol extract of leaves of Clerodendron infortunatum (Linn.) were collected from West Bengal, India for anti-inflammatory activity against carrageenan, histamine and dextran induced rat paw edema. The methanol extract (250 and 500mg/kg b.wt.) exhibited significant activity (p<0.01) against all phlogistic agent used in dose dependant manner. All these effects was compared with reference drug phenylbutazone (Das et al., 2010).

The methanolic extract of dried leaves of Aleuritis moluccana (AMME) were collected from Punjab, India was investigated for anti-inflammatory (carrageenan induced rat paw oedema) and anti-pyretic (brewer’s yeast induced pyrexia) activities. Pre treatment with extract (100-300mg/kg p.o.) significantly prevented increase in volume of paw oedema in dose dependent manner. A maximal effect was observed at 300mg/kg which was comparable to diclofenac sodium 20mg/kg orally. Ceiling effect at the dose of 300mg/kg was observed. The antipyretic effect of Aleuritis moluccana (measured as % reduction in body temperature) was compared with paracetamol 150mg/kg orally. Aleuritis moluccana in dose of 300mg/kg caused significant decrease in body temperature of rats (Niazi et al., 2010).

Coriandrum sativum, Datura stramonium and Azadirachta indica were collected from Bhopal are traditionally used in treatment of inflammation. Ethanolic extracts of fruits of Coriandrum sativum, leaves of Datura stramonium and Azadirachta indica were subjected to preliminary screening for anti-inflammatory activity in albino rats. All
ethanolic extracts exhibited significant anti-inflammatory activity comparable to the standard drug diclofenac sodium against carrageenan induced rat paw edema method. Among these plant *Azadirachta indica* showed maximum anti-inflammatory activity every hour (Gupta et al., 2010).

The effects of *Cordia dichotoma* forst f. seeds extracts were collected from Etawah, Uttar Pradesh, India on different phases of acute inflammation was examined. Investigations were performed using different phlogistic agents induced paw edema viz., carrageenan induced paw oedema and dextran induced paw oedema in rats. Various extracts (ethanol and aqueous) of *Cordia dichotoma* forst seeds at a dose of 250mg/kg and 500mg/kg orally were tested. Diclofenac sodium at the dose of 10mg/kg was used as standard. Both extracts showed significant activity (*p<0.05 and **p<0.01) compared with control in both of these models (Sharma et al., 2010).

### 2.4 Anti-hyperglycemic activity of medicinal plants

Diabetes mellitus is a disease in which homeostasis of carbohydrates, protein and lipid metabolism is improperly regulated by the insulin resulting in elevation of fasting and post prandial blood glucose levels (Tiwari and Rao, 2002). Diabetes mellitus is heterogeneous disorder with multiple etiological factors; it generally involves absolute or insulin resistance.

Insulin is a hormone that is central to regulating the energy and glucose metabolism in the body. Insulin caused cells in the liver, muscle and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle. The modern insulin were obtained from pork and beef and have eliminated antigenic potential of the earlier preparation (Federlin et al., 1981). When control of insulin levels fails, diabetes mellitus would result and caused atrophy at the site of injection (Reeves et al., 1980).

Sulfonylurea (UK: Sulphonylurea) derivatives are a class of antidiabetic drugs that are used in the management of diabetes mellitus type 2 ("adult-onset"). They act by increasing insulin release from the beta cells in the pancreas. The choice depends on the propensity of the patient to develop anti-hyperglycemia long acting sulfonylureas with active metabolites can induced anti-hyperglycemia. They can, however, help achieve glycemic control when tolerated by the patient. The shorter acting agents may not control blood sugar levels adequately (Kunte et al., 2007).
Blood glucose levels of normal and alloxan diabetic rabbits was determined after oral administration of various doses of *Achyranthes aspera* powdered whole plant and certain aqueous and methanolic extracts. Oral administration of 2, 3 and 4gm/kg of *Achyranthes aspera* powder produced significant dose related hypoglycemic effect in normal as well as in diabetic rabbits. The water and methanol extracts also decreased blood glucose levels in normal and alloxan diabetic rabbits. A 7 day acute toxicity study in rabbits did not reveal any adverse or side effects of this folk medicine at dosages up to 8gm/kg orally. It is possible plant could act by providing certain necessary elements like calcium, zinc, magnesium, manganese and copper to the beta-cells (Akhtar and Iqbal, 1991).

The effect of shilajit (a herbomineral preparation) on blood glucose and lipid profile in euglycemic and alloxan induced diabetic rats. Diabetes was induced in albino rats by administration of a single dose of alloxan monohydrate 5% (125mg/kg i.p.). Effects of three different doses of shilajit (50, 100 and 200mg/kg/day, orally), alone for 4 weeks and a combination of shilajit (100mg/kg/day, orally) with either glibenclamide (5mg/kg/day, orally) or metformin (0.5gm/kg/day, orally) for 4 weeks was studied on blood glucose and lipid profile. In the diabetic rats, all the three doses of shilajit produced significant reduction in blood glucose levels and also produced beneficial effects on the lipid profile. The maximum effect was observed with (100mg/kg/day) dose of shilajit. Combination of shilajit 100mg/kg with glibenclamide (5mg/kg/day) or metformin (0.5gm/kg/day) significantly enhanced glucose lowering ability and improvement in lipid profile than any of these drugs given alone (Trivedi et al., 2004).

Aqueous extracts of the seeds of fenugreek (*Trigonella foenum graecum*) and fruits of bitter gourd (*Momordica charantia*) were tested for hypoglycemic activity. Daily administration of *Trigonella foenum graecum* for 7 days failed to induced any significant change in the blood glucose levels. However, an extended 15 day treatment regimen was found to significantly reduced the blood glucose levels. The blood glucose levels was found to decrease at 6 and 9h after the final administration of the extract. In contrast, both 7 and 15 days daily administration of *Momordica charantia* reduced blood glucose levels drastically (p<0.05 and p<0.01, respectively). Significant reduction in blood glucose levels was observed at 6 and 9h after the short term treatment and at 3, 6 and 9h after the long term treatment. Long term administration of both *Trigonella foenum graecum* and
Momordica charantia was found to decrease blood glucose levels during Oral Glucose Tolerance Test at 30, 60 and 90min (Srinivasan and Karundevi, 2005).

Hypoglycemic activity of aqueous leaf extract of Persea americana Mill. were collected from Nigeria revealed extract contained various pharmacologically active compounds such as saponins, tannins, phlobatannins, flavonoids, alkaloids, and polysaccharides. The administration of aqueous extract of Persea americana 100-200mg/kg to alloxan diabetic rats produced significant reduction (P<0.01) in blood glucose level in a dose dependent fashion after a single dose of the extract, as well as following prolonged treatment 7 days compared to the control group. Maximum antidiabetic activity was reached at 6h after a single dose of the extract, producing 60.02 ± 6.83% reduction in blood glucose levels. However, the hypoglycemic effect of the extract was incomparable to the reference drug (chlorpropamide). The extract 100-200mg/kg produced sustained significant (P<0.01) antidiabetic activity during prolonged treatment 7 days. A sustained significant (P<0.01) reduction in blood glucose levels of the treated rats was observed throughout the period of treatment. The hypoglycemic effect was not comparable to the reference group (Anita et al., 2005).

The antidiabetic activity of Ginkgo biloba was evaluated on streptozotocin induced diabetes in albino wistar rats. Ginkgo biloba in a high dose of 100mg/kg produced significant reduction in fasting blood sugar of 31% (Shankar et al., 2005).

Antidiabetic activity of ethanolic leaf extract of Croton zambesicus Muell. Arg. from Nigeria was evaluated using alloxan induced 150mg/kg hyperglycemic rats. The activity of the ethanolic leaf extract was compared with reference drug chlorpropamide. The extract produced significant (P<0.01) reduction in blood glucose level after a single dose of the extract and in prolonged treatment for 7 days. The antidiabetic activity was comparable to the reference drug chlorpropamide (Okokon et al., 2006).

The antidiabetic activity of the fruits of Momordica dioica by preparing the various organic extracts were collected from Andhra Pradesh, India and the resultant extracts screening for the antidiabetic activity to track the activity. The chloroform, ethylacetate and alcohol extracts of Momordica dioica fruits were made and subjected for phytochemical screening and tested for their antidiabetic activity in alloxan induced diabetic rats. Phytochemical screening showed positive test for steroids and/or triterpenoids (CHCl₃ extract), steroids and/or triterpenoids and their glycosides (EtoAc
extract), steroids and/or triterpenoids and their glycosides (EtOH extract). The ethyl acetate and ethanol have been shown significant antidiabetic activity at a dose of (200mg/kg p.o.) (Reddy et al., 2006).

*Terminalia chebula (T. chebula)* fruits extract from Tamilnadu, India were screened for antidiabetic activity on streptozotocin (STZ) induced experimental diabetes in rats. Oral administration of ethanolic extract of the fruits (200mg/kg b.wt./rat/day) for 30 days significantly reduced levels of blood glucose and glycosylated haemoglobin in diabetic rats. Determination of plasma insulin levels revealed insulin stimulating action of the fruit extract. Also, the alterations observed in the activities of carbohydrate and glycogen metabolising enzymes were reverted back to near normal after 30 days of treatment with extract. Electron microscopic studies showed significant morphological changes in the mitochondria and endoplasmic reticulum of pancreatic β cells of STZ induced diabetic rats. Also, a decrease in the number of secretory granules of β cells was observed in the STZ induced diabetic rats and a these pathological abnormalities were normalized after treatment with *Terminalia chebula* extract. The efficacy of the fruit extract was comparable with glibenclamide, a well known hypoglycemic drug (Kumar et al., 2006).

The ethanol extract of *Passiflora mollissima* were tested for its anti diabetic activity in alloxan induced diabetic rats. The extract was studied at two dose level, 100mg/kg and 200mg/kg respectively. The activity was compared with reference standard, phenformin and control. The plant extract at a dose of 100mg/kg and 200mg/kg significantly (P<0.001) lowered blood sugar level of hyperglycemic rats (Edwin et al., 2007).

The effect of the methanol leaf extract, dichloromethane, ethylacetate and n-hexane fractions from *Anacardium occidentale* (Linn.) were collected from Lolodorf (South Province, Cameroon) studies for antidiabetic activity in streptozotocin induced diabetic rats. Oral administration of methanol extract at doses of 35, 175 and 250mg/kg significantly reduced blood glucose levels in diabetic rats 3 hours after administration. Of three different doses, maximum reduction in 37% and 35% in blood glucose levels was respectively observed with doses of 175 and 250mg/kg. When administered repeatedly, the blood glucose reducing effect of the methanol extract at the dose of 175mg/kg in
diabetic rats became more pronounced 48%. Fractions from the methanol extract at the dose of 175mg/kg also decreased blood glucose levels in diabetic rats after repeated administration. The n-hexane fraction produced maximum hypoglycemic effect 45% and the same dose of the dichloromethane and ethylacetate fractions respectively reduced hyperglycemia by 21% and 41% at the end of the treatment. On the other hand, a significant decrease in urine glucose levels was observed in diabetic rats after repeated administration of the methanol extract and fractions (Sokeng et al., 2007).

*Cissus verticillata* leaves extract from Brazil (Methanol soluble fraction) (MSF) as well as that of tyramine (TYR) of *Cissus verticillata* was investigated, for antidiabetic activity in the model of alloxan induced diabetes in male wistar rats. The MSF (50 and 100mg/kg p.o.), tyramine (1 and 2mg/kg p.o.), glibenclamide (GLI 5mg/kg p.o.) and metformin (MET 50mg/kg p.o.) were administered daily for 5 days, 48h after the alloxan injection 40mg/kg. Drugs were administered alone or associated. These results point to the insulin like effects of *Cissus verticillata*, probably due to the presence of tyramine in the plant (Lino et al., 2007).

Evaluation of hypoglycemic activity were tested for methanolic stem bark extract of *Adansonnia digitata* from Nigeria in wistar rats. Antidiabetic potentials of the plant extract at the doses of 100, 200 and 400mg/kg intraperitoneally administered on streptozotocin diabetes wistar rats. Treatment of streptozotocin diabetic wistar rats with extract caused significant (P<0.05) reduction in blood glucose levels when compared with control. The dose of 100mg/kg have been shown a significant decreased (p<0.05) after 1, 3, 5 and 7 hours of extract administration when compared to control normal saline. Also the dose of 200mg/kg have been shown a significant decreased (p<0.05) after 3, 5 and 7 hours of extract administration. The dose of 400mg/kg have been shown a significant decreased (p<0.05) after 5 and 7 hours of extract administration when compared to control normal saline. The highest activity resides at the dose of 100mg/kg with percentage glycemic change of 51% after 7 hours of extract administration while other two doses 200 and 400mg/kg have glycemic change of 39% and 31% respectively after 7 hours of extract administration. The phytochemical screening revealed presences of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids. The median lethal dose (LD₅₀) in mice was calculated to be (1264.9mg/kg b.wt.) (Tanko et al., 2008).
Aloe vera (Linn.) Burm. fil. was evaluated for antidiabetic activity in streptozotocin induced diabetic rats. In diabetic induced rats fed with Aloe vera (300mg/kg b.wt.), the fasting plasma glucose levels was reduced to normal and body weight was found to be increased. In the pancreatic sections of diabetic rats fed with Aloe vera, the islets was comparable to normal rats. In liver, the changes caused after induction of diabetes are granular cytoplasm, dilated sinusoids, shrunken nuclei and inflammation, which was reduced after feeding with Aloe vera. Excess proliferation of epithelium in the small intestine was observed in diabetic rats, which was reduced after Aloe vera feeding. In diabetic rats fed with Aloe vera no change was noticed in the kidney and stomach (Noor et al., 2008).

Petroleum ether (60°-80° C), chloroform, acetone, ethanol, aqueous and crude hot water extracts of the whole plant of Cynodon dactylon from Tamilnadu, India were tested for anti-hyperglycemic activity in glucose overloaded hyperglycemic rats and in alloxan induced diabetic model at two dose levels (200 and 400mg/kg p.o.) respectively. The aqueous extract of Cynodon dactylon and the non polysaccharide fraction of aqueous extract was found to exhibited significant anti-hyperglycemic activity and only the non polysaccharide fraction was found to produce hypoglycemia in fasted normal rats. Treatment of diabetic rats with aqueous extract and non polysaccharide fraction of the plant decreased elevated biochemical parameters, glucose, urea, creatinine, serum cholesterol, serum triglycerides, high density lipoprotein, low density lipoprotein, haemoglobin and glycosylated haemoglobin significantly. Comparatively, the non polysaccharide fraction of aqueous extract was found to be more effective than aqueous extract (Jarald et al., 2008).

A number of potential antidiabetic plants were identified through an ethnobotanical survey of the traditional pharmacopeia of the Cree of Eeyou Istchee (CEI–Northeastern Canada) used against symptoms of diabetes and their biological activity assessed by in vitro bioassays. Among these, Sorbus decora C.K. Schneid. (Rosacea) ranked highly and increased transport of glucose in skeletal muscle cells in culture. Antidiabetic potential of Sorbus decora in in vivo models of insulin resistance and diabetes notably the streptozotocin Type 1 diabetic rat (STZ), the genetic KK-A^y Type 2 diabetic mouse and the rat rendered insulin resistant with 10% glucose water consumption for 6
weeks. The ethanol extract of *Sorbus decora* demonstrated both anti-hyperglycemic and insulin sensitizing activity *in vivo*, thereby confirming antidiabetic potential and validating CEI traditional medicine (Vianna et al., 2009).

Aqueous extract of leaves of 3 herbs (*Murraya koenigii*, MK; *Psidium guajava*, PG and *Catharanthus roseus*, CR) were collected from Indore, India was used to test their antidiabetic activity in streptozotocin (STZ) induced diabetic albino rats. *Murraya koenigii, Psidium guajava* and *Catharanthus roseus* are given to the STZ induced diabetic rats at the concentration of (500mg/kg b.wt.) in different groups of 6 diabetic rats each orally once a day for 15 days. Glibenclamide (GBC) is also given to another group to support results at the concentration of (3mg/kg b.wt.) orally once a day for 15 days. Diabetic control received vehicle. Body weight showed significant increase (*Murraya koenigii* and *Psidium guajava* p<0.05, *Catharanthus roseus* and GBC: p<0.001) after 15 days of treatment with herbal extract when compared with control. Blood glucose level on 15th day of treatment became significantly low (p<0.001). At the termination of the experiment on 15th day the urine glucose and ketone were absent in herbal treated group which was present in the diabetic control. The aqueous extract of leaves of *Murraya koenigii, Psidium guajava* and *Catharanthus roseus* at the dose of (500mg/kg b.wt.) brings about significant beneficial effects in various physiological/histological parameters altered during diabetic manifestations and these effects are quite comparable with glibenclamide (a standard drug used to treat diabetes mellitus) (Prasad et al., 2009).

*Strophanthus hispidus* Del. a plant widely distributed throughout the open African savanna, is used by ethnic, tribal people of Africa for various purposes, including the control of diabetes. The phytochemical screening indicated presence of alkaloids, flavonoids, saponins, and cardiac and cyanogenic glycosides. Significant concentration dependent hypoglycemic effects (p<0.05) of both ethanol and chloroform extracts was observed with 50% extracts being more effective than 20% extracts. The ethanol leaf extract showed higher hypoglycemic effect 60% than other extracts at 1 hour after extract administration. The 50% and 20% chloroform root extracts exhibited more prolonged hypoglycemic effects than those of other chloroform and ethanol extracts. The highest hypoglycemic activity was observed at 1.5 hours after of extract administration, with an
increase in blood glucose concentrations noted by 2 hours after extract administration (Ojiako and Igwe, 2009).

Effects of ethanolic extracts of alligator pear seed (*Persea americana*) were collected from Nigeria on normal and alloxan-induced diabetic rats were investigated in 6 groups of rats (5 rats per group). Test groups were made diabetic with intra-peritoneal injection of alloxan and treated with (450mg and 900mg/kg b.wt.) of alligator pear seed extract. Two non-diabetic groups were also administered with (450mg and 900mg/kg b.wt.) extract. The levels of blood glucose were examined in all 6 experimental groups. In diabetic rats, blood glucose levels were significantly reduced (p<0.01) by 46.60-55.14% on consumption of the extracts, with greater effect exhibited by the 900mg/kg extract. In non-diabetic rats, blood glucose levels were reduced by 14.62-17.33% on consumption of the seed extract. Histological studies showed a degenerative effect on the pancreatic islet cells of diabetic rats (Edem, 2009).

Effect of methanolic extract of *Musa sapientum* (Linn.) Sucker was evaluated for antidiabetic activity on fasting blood glucose, body weight and pancreas histology of alloxan induced hyperglycemic rats and compared with reference drug, glibenclamide. The extract at all tested doses (5mg and 10mg/kg/day) significantly (p<0.05) lowered fasting blood glucose level in the treated rats compared with diabetic but untreated rats (test control). At 10mg/kg administration of the extract, a significant increase in body weight was observed compared with diabetic untreated group. The pancreas histology indicates significant recovery with extract administration. The efficacy of the extract in reducing blood glucose level, improving body weight and rejuvenating the damaged pancreas of alloxan induced diabetic rats favorably compared with reference drug, glibenclamide (Salau et al., 2010).

*Crateva nurvala* stem bark extracts were collected from Karnataka, India (Capparidaceae) was investigated for antidiabetic activity in alloxan induced diabetic albino rats. A comparison were made between the action of different extracts of *Crateva nurvala* and a known antidiabetic drug glibenclamide (600μg/kg b.wt.). The petroleum ether, chloroform, alcohol, and aqueous extracts of *Crateva nurvala* stem bark were obtained by simple maceration method and were subjected to standardization. Dose selection were made on the basis of acute oral toxicity study (50-5000mg/kg b.wt.).
*Crateva nurvala* petroleum ether extract (CNPEE) and ethanolic extract (CNEE) showed significant (P<0.001) antidiabetic activities (Sikarwar and Patil, 2010).

Dried petroleum ether (60°-80° C) extracts of flower head of *Sphaeranthus indicus* from Lalitpur, Uttar Pradesh, India were subjected for hypoglycemic activity in wistar rats 150-200gm. The oral administration of flower head extracts at doses of 200mg/kg lead to significant blood glucose reduction (Jha et al., 2010).

*Ficus glomerata* (Linn.) was undertaken to screen hypoglycemic activity of ethanol extracts of leaves of *Ficus glomerata*. The results showed significant anti-hyperglycemic effect in experimental model of diabetes mellitus (Sharma et al., 2010).

### 2.5 Hypolipidemic activity of medicinal plants

The increased risk for premature arteriosclerosis due to increased in triglycerides and low density lipoprotein levels. Hyperlipidemia is an independent predictor of high risk for Coronary heart disease (CHD). About 70-80% of deaths in patients are due to vascular disease. An ideal treatment hyperlipidemia would be a drug that are not only controls but also prevents development of arteriosclerosis (Halliwell and Gutteridge, 1985).

The hypolipidemic effects of *Lycium barbarum* fruit water decoction, crude polysaccharide extracts (crude LBP), and purified polysaccharide fractions (LBP-X) from China was investigated in alloxan induced diabetic or hyperlipidemic rabbits through serum lipid parameters. It was found that three *Lycium barbarum* fruit extracts/fractions could significantly reduced blood glucose levels and serum total cholesterol (TC) and triglycerides (TG) concentrations and at same time markedly increase high density lipoprotein cholesterol (HDL-C) levels after 10 days treatment in tested rabbits, indicating there were substantial hypolipidemic effects (Luo et al., 2004).

Indian black tea were collected from Kolkata, India in normal as well as triton wr-1339 induced hyperlipidemic rats caused decrease in their plasma levels of total cholesterol, phospholipids and triglycerides (Chander et al., 2005).

The effect of methanolic leaves extract of *Achyranthes aspera* on lipid profile in female rats was evaluated using electrochemiluminescence immunoassay. The extract, however, did not significantly influence serum concentration of the ovarian hormones and various lipids except lowering HDL at doses tested (Shibeshi et al., 2006).
Globe artichoke (*Cynara scolymus*) is a vegetable with medicinal value. The globe artichoke was processed to made into powder. The acceptability study of the five developed recipes revealed all the recipes were acceptable with wheat biscuits scoring highest score followed by groundnut biscuits and wheat bread. Administration of 6gm of globe artichoke powder in the form of biscuits caused statistically significant reductions in blood glucose levels and serum lipid profile values (Nazni et al., 2006).

To consider potentially new hypoglycemic and anti-hyperlipidemic sources, aqueous extracts from four Korean medicinal plants, *Chrysanthemum coronarium*, *Dioscorea batatas*, *Morus alba* and *Citrus unshiu*, was investigated in alloxan induced diabetic rats. There was no significant difference in triglycerides (TG) between the diabetic control and diabetic rats treated, respectively, with extracts of *Chrysanthemum coronarium*, *Dioscorea batatas*, *Morus alba* and *Citrus unshiu*. The administration of *Citrus unshiu* showed more anti-hyperlipidemic effect (Kim et al., 2006).

*Ficus racemosa* (Moraceae) is used in traditional system of medicine for the treatment of several disorders including diabetes mellitus. To investigated hypolipidemic activities of ethanolic extract of *Ficus racemosa* bark in alloxan induced diabetic rats. An increase in blood glucose was accompanied by an increase in total cholesterol, phospholipids, triglycerides, FFA and decrease in HDL cholesterol in diabetic rats. Oral administration of ethanolic extract of *Ficus racemosa* bark (300mg/kg b.wt.) to diabetic rats restrored status of blood glucose, lipids and lipoproteins to near normal range. Ethanolic extract of *Ficus racemosa* bark have potent hypolipidemic effects in alloxan induced diabetic rats and these effects were much comparable to the standard reference drug, glibenclamide (Sophia and Manoharan, 2007).

The aqueous extract of the leaves of *Eclipta prostrata* from Tamilnadu, India showed significant reduction in total cholesterol, triglycerides, total protein and elevation in high density lipoprotein cholesterol. The aqueous extract of *Eclipta prostrata* was found to possess significant hypolipidemic activity. The results also suggest *Eclipta prostrata* leaf extract at (100 and 200mg/kg b.wt.) concentrations is an excellent lipid lowering agent (Dhandapani, 2007).

Ischemic heart disease (IHD) is a leading caused of morbidity and mortality in both developing and developed countries. An underlying caused of Ischemic heart
disease involves retention and deposit of serum lipids in coronary arteries, decreasing blood flow. Drugs (conventional and herbal) are used to lower levels of serum cholesterol to help prevent Ischemic heart disease. The Ayurvedic medicine pharmacopoeia identified herbs that might contribute to a decrease in cholesterol and therefore reduced the risk of Ischemic heart disease. Additional assessments of safety using a scale and determination of reported efficacy/effectiveness of the randomized controlled trials (RCTs). (RCTs) generally received high quality scores and improved by decade of publication. More than 50% of Garlic, more than 80% of Guggul, and 100% of Arjuna (RCTs) reported product effectiveness. Safety scores did not improve by decade (Singh et al., 2007).

Hypolipidemic activity of fresh grass juice of *Triticum aestivum* was evaluated in normal rats. The freshly prepared *Triticum aestivum* grass juice were administered to normal rats at the dose of 5ml/kg and 10ml/kg orally once daily for 21 days. Blood samples were collected after 24 hours of last administration and used for estimation of lipid profile. Fresh grass juice were also subjected to preliminary phytochemical screening. Fresh grass juice administration produced dose related significant (P<0.05) reduction in total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol levels in normal rats as compared to control. Preliminary phytochemical screening revealed presence of alkaloids, tannins, saponins and sterols in *Triticum aestivum* grass (Kothari et al., 2008).

Extracts from *Abelmoschus esculentus* are known to ameliorate hyperlipidemia in diabetic mice induced by alloxan and streptozotocin. Hypolipidemic activity of the extracts from total plant by dichloromethane (AE1) and methanol (AE2), and from fruit by dichloromethane (AE3) and methanol (AE4) was studied and compared with simvastatin (Zocor®). Hyperlipidemia in mice was induced by single intraperitoneal injection of 300mg/kg of tyloxapol. Studied extracts were orally administered at dose equivalent to 30gm of dry extract/kg immediately after tyloxapol injection. Cholesterol levels decreased 56.45%, 55.65%, 41.13%, 40.50% and 53.63%, respectively in groups orally administered AE1, AE2, AE3, AE4 and simvastatin as compared to the tyloxapol injected group (Ngoc et al., 2008).
The effect of various extracts 50% ethanolic of *Capparis decidua* on lipid profile of streptozotocin diabetic rats was studied from samples were collected from Jodhpur. The extract were administered to the diabetic models for 30 days. The extract produced significant (p<0.05) dose dependent decrease in the levels of total cholesterol (TC), triacylglycerol (TG), low density lipoprotein cholesterol (LDL-C), with significant increase in the level high density lipoprotein cholesterol (HDL-C). The extracts of *Capparis decidua* prove to have hypolipidemic potential (Chahlia, 2009).

The effect of gum ghatti of *Anogeissus lalifolia* from Karnataka, India on serum lipid levels was investigated in albino rats. Rats were made hyperlipidemic by the oral administration of cholesterol (400mg/kg b.wt./day) along with cholic acid 50mg/kg in coconut oil. The hypolipidemic effect was compared with control. The rats was divided into six groups of six animals each. In atherogenic diet induced hyperlipidemic model, the rats receiving treatment with gum ghatti at 250mg/kg dosage showed significant reduction in serum triglycerides (82.75 ± 0.63) only and there was no significant changes either in serum total cholesterol or elevation in HDL where as, at 500 and 750mg/kg dosage showed significant reduction in serum total cholesterol (72.85 ± 0.60, 68.17 ± 0.95) and serum triglycerides (78.92 ± 0.34, 75.93 ± 1.05). Further, the 750mg/kg dose have also exhibited significant elevation in high density lipoprotein cholesterol (41.13 ± 0.37) (Parvathi et al., 2009).

The anti-hyperlipidemic effect of plant *Phyllanthus amarus* Schumach from Maharashtra, India was evaluated against cholesterol diet induced hyperlipidemia in wistar rats. Hydro alcoholic extract of leaves of *Phyllanthus amarus* Schumach was studied for its *in vivo* anti-hyperlipidemic potential using cholesterol diet induced hyperlipidemia model in rats. The result indicated *Phyllanthus amarus* Schumach possess significant hypolipidemic activity at doses 300 and 500mg/kg (Umbare et al., 2009).

Based on high saponin content in herbal plants, *Sapindus emarginatus* (SE) from Tamilnadu, India was evaluated for anti-hyperlipidemic activity of methanol extract of pericarps of *Sapindus emarginatus* against triton induced hyperlipidemia in rats. *Sapindus emarginatus* were administered at a dose of (100 and 200mg/kg p.o.) to triton induced hyperlipidemic rats. Fenofibrate was used as reference standard. *Sapindus emarginatus* showed significant decrease in the levels of serum cholesterol,
phospholipids, triglycerides, LDL, VLDL and significant increase in the level of serum HDL at the dose of (100 and 200mg/kg p.o.) against triton induced hyperlipidemic in rats. Methanol extracts decreased serum level of total cholesterol by 69.72%. On the other hand aqueous extract of Sapindus emarginatus increased serum HDL cholesterol level by 24.11%. The reduction in LDL cholesterol level by extract was 30.31% (Jeyabalan and Palayan, 2009).

Hypolipidaemic activity of Rauwolfia serpentina Benth. was investigated and were collected from Karachi, Pakistan to provide a base for isolation of active principle and to validate its use for the control of hyperlipidaemia, the major caused of cardiovascular diseases prevailing worldwide. Twelve days trial was done with oral administration of root powder of Rauwolfia serpentina 30mg/kg and distilled water 1ml/kg in test and control rabbits respectively. The blood were collected from each group on 1st, 4th, 8th and 12th day to estimate serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). Gradual decrease observed in TG and LDL-C levels from 204.76-111.94 and 33.31-10.85mg/dl, respectively in test when compared with control rabbits (p<0.0001). Similarly, TC were significantly decreased when compared 1st-12th day of treatment in test group (p<0.0001) and test on 12 day compared with control (p<0.05). However, HDL-C remained constant in test rabbits but found higher than control (p<0.05). ALT and LDH levels was found normal. According to the data, root powder of Rauwolfia serpentina have hypotriglyceridemic and hypocholesterolemic effects with undetectable side effects on liver and cardiac functions (Qureshi and Udani, 2009).

The lipid lowering effects of low esterified pectins from the sea grass Zostera marina and citrus fruits from Sea of Japan was investigated in animals with ethanol intoxication. Regardless of their source, pectins with low degree of esterification contributed to reduced cholesterol and triglycerides levels, both in the blood and liver, and enhanced blood high density lipoprotein level, as well as slowing lipid peroxidation processes (Khotimchenko, 2009).

The effect of 50% ethanolic extract of Cassia fistula (Linn.) (Fabaceae) legume were collected from Rajasthan, Jaipur, India was assessed on serum lipid metabolism in
cholesterol fed rats. Oral feeding of cholesterol (500mg/kg b.wt./day) dissolved in coconut oil (0.5ml/rat/day) for 90 days caused significant (P<0.001) elevation in total and LDL-cholesterol, triglycerides and phospholipids in serum of rats. Administration of *Cassia fistula* legume extract at the doses (100, 250 and 500mg/kg b.wt./day) along with cholesterol significantly prevented rise in the serum total and LDL-cholesterol, triglycerides and phospholipids in a dose dependent manner. The ratio of HDL-cholesterol/total cholesterol ratio was elevated in serum of *Cassia fistula* extract treated groups as compared to cholesterol alone fed control rats (Gupta and Jain, 2009).

The hypolipidemic activity of high flavonoid containing herbal, *Sesbania grandiflora* (SG) from Tamilnadu, India studied for anti-hyperlipidemic activity of aqueous extract of leaves of *Sesbania grandiflora* against triton induced hyperlipidemia in rats. *Sesbania grandiflora* administered a dose of (200μg/kg p.o.) to the triton induced hyperlipidemic rats. *Sesbania grandiflora* showed significant decrease in the levels of serum cholesterol, phospholipids, triglycerides, LDL, VLDL and significant increase in the level of serum HDL at the dose of (200μg/kg p.o.) against triton induced hyperlipidemic in rats. Aqueous extract of leaves of *Sesbania grandiflora* was investigated hypolipidemic activity on triton induced hyperlipidemic profile. Aqueous extract fraction decreased serum level of total cholesterol by 69.72%. On the other hand, aqueous extract of *Sesbania grandiflora* increased serum HDL cholesterol level by 24.11%. The reduction in LDL cholesterol level by aqueous extract was 30.31% (Saravanakumar et al., 2010).

The lipid lowering activity of *Anthocephalus indicus* (Rubiaceae; Hindi name Kadamba) root extract were collected from Lucknow in triton wr-1339 induced hyperlipidemia in rats. In this model, feeding with root extract (500mg/kg b.wt.) lowered plasma lipids and reactivated post-heparin lipolytic activity in hyperlipidemic rats. Furthermore, the root extract (50-500μm) inhibited the generation of superoxide anions and hydroxyl radicals, in both enzymic and non-enzymic systems, *in vitro*. The results demonstrated both lipid lowering and antioxidant activities in root extract of *Anthocephalus indicus*, which could help prevention of hyperlipidemia and related diseases (Kumar et al., 2010).
Haritaki (*Terminalia chebula*) (Combretaceae) were collected from Bangalore, India and an important plant used traditionally for medicinal purposes. It is component of the classic Ayurvedic combination called Triphala. Hyperlipidemia was induced by treated orally with atherogenic diet. In atherogenic diet induced hyperlipidemic model, the rats receiving treatment with Haritaki showed significant reduction in total cholesterol, triglycerides, total protein and elevation of high density lipoprotein cholesterol. Haritaki was found to possess significant hypolipidemic activity. The results also suggest Haritaki at (1.05 and 2.10 mg/kg b.wt.) concentrations are an excellent lipid lowering agent (Maruthappan and Shree, 2010).

In *vitro* and hypolipidemic activity of the total flavonoids (TFs) from the *Rosa laevigata* Michx fruit from China was evaluated, in hyperlipidemic mice were intragastric administrated with TFs (25 and 50mg/kg/day) for 4 weeks, and fenofibrate was used as the positive reference substance. After the experiment, the levels of TC (total cholesterol), TG (triglycerides), LDL-C (low density lipoprotein cholesterol) of the mice administrated with high dose of TFs were markedly declined by 45.02%, 33.86% and 73.68%, respectively while HDL-C (high density lipoprotein cholesterol) were significantly increased compared with model group (Liu et al., 2010).

The hypolipidemic activity of *Semecarpus anacardium* was evaluated in male wistar rats weighing 250-270gm were injected with streptozotocin at a dose of (50mg/kg b.wt.) and administered with *Semecarpus anacardium* (300mg/kg b.wt.) and metformin (500mg/kg b.wt.) for 21 days. After the experimental duration, serum were collected, liver and kidney were excised and used for the analysis of lipid and lipid metabolizing enzymes. The results revealed *Semecarpus anacardium* administration was able to decrease levels of LDL, cholesterol, VLDL, TG, phospholipids and free fatty acid and increase the HDL levels and favorably modulate the lipid metabolizing enzymes in the liver and kidney. These results express *Semecarpus anacardium* revealed hypolipidemic activity in diabetic rats (Jaya et al., 2010).

### 2.6 Antitumor activity of medicinal plants

Cancer is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent
tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood) (Kinzler et al., 2002).

Cancers are classified by the type of cell that resembles the tumor and, therefore, the tissue presumed to be the origin of the tumor. Examples of general categories include:

Carcinoma: Malignant tumors derived from epithelial cells. This group represents the most common cancers, including the common forms of breast, prostate, lung and colon cancer (Nelson et al., 2004).

Sarcoma: Malignant tumors derived from connective tissue or mesenchymal cells.

Lymphoma and leukemia: Malignancies derived from hematopoietic (blood-forming) cells (Sasco et al., 2004).

Germ cell tumor: Tumors derived from totipotent cells. In adults most often found in the testicle and ovary; in fetuses, babies, and young children most often found on the body midline, particularly at the tip of the tailbone (Biesalski et al., 1998).

Many people use traditional medicine as an alternative treatment for cancer. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents.

*Achyranthes aspera* leaves have been assessed for chemopreventive activity. The MeOH extract, alkaloid, non-alkaloid and saponins fractions exhibited significant inhibitory effects (concentration 100μg) on the Epstein-Barr virus early antigen activation induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate in Raji cells. In this *in vitro* assay the non-alkaloid fraction containing mainly non polar compounds showed most significant inhibitory activity (96.9%; 60% viability). In the *in vivo* two stage mouse skin carcinogenesis test the total methanolic extract possessed pronounced anticarcinogenic effect 76%. *Achyranthes aspera* leaf extract and the non-alkaloid fraction are valuable antitumor promotors in carcinogenesis (Chakraborty et al., 2002).

One hundred and thirty endophytic fungi isolated from 12 Chinese traditional medicinal plants were collected from Yuanmou county and Dawei Mountain, Yunnan Province, Southwest China, were tested for antitumour activities by MTT assay on human gastric tumour cell line (BGC-823) and the growth inhibition test against 7 phytopathogenic fungi. The results showed fermentation broths from 9.2% of the isolates
exhibited antitumour activity and 30% exhibited antifungal activity. The results indicate endophytic fungi of Chinese traditional medicinal plants are promising sources of novel bioactive compounds (Li et al., 2005).

Resveratrol (3, 4', 5-trihydroxy-trans-stilbene), a naturally occurring phytoalexin found in grapes and wine, possesses cancer preventive activity. Angiogenesis is a crucial step in the growth and metastasis of cancers. The effect of resveratrol on angiogenesis was studied in vitro and ex vivo, and found that resveratrol directly inhibited human umbilical vein endothelial cell growth and decreased gelatinolytic activities of matrix metalloproteinase-2. Tube formation was inhibited by treatment with resveratrol after plating endothelial cells on matrigel. Resveratrol treatment also inhibited endothelial cell attachment to basement membrane components fibronectin and laminin, and displays similar behavior on cell chemotaxis. In addition, resveratrol have been found to be an angiogenesis inhibitor in the rat aorta matrix culture model. Therefore, inhibition of angiogenesis associated with cancer may be a novel mechanism for the anticancer activity of resveratrol (Cao et al., 2005).

Twelve Thai medicinal plants as the ingredients of a Southern Thai traditional formula for cancer treatment were selected to test cytotoxicity activity against two types of human cancer cell lines; large cell lung carcinoma (CORL-23) and prostate cancer cell lines (PC3) and one type of normal human cell line, fibroblast cells (10FS). SRB assay was used to test cytotoxic activity against all the cell types. The ethanolic extracts of six plants (Bridelia ovata, Curcuma zedoaria, Derris scandens, Dioscorea membranacea, Nardostachys jatamansi and Rhinacanthus nasutus) showed cytotoxic activity (IC$_{50}$ < 30 μg/ml) against lung and prostate cancer cell lines. Dioscorea membranacea root showed highest cytotoxic activity against lung cancer cell lines (IC$_{50}$ = 4.6μg/ml) but it exhibited low cytotoxic activity against prostate cancer cell lines (IC$_{50}$ = 17.55μg/ml) and less cytotoxic activity against normal cell lines (IC$_{50}$ = 66.05μg/ml). Curcuma zedoaria showed cytotoxic activity against CORL-23 and PC3 but less cytotoxic activity against 10FS (IC$_{50}$ = 6.05, 17.84 and 55.50μg/ml respectively). Rhinacanthus nasutus root extract showed highest cytotoxic activity against PC3 (IC$_{50}$ = 2.01μg/ml) and this extract also showed high activity against CORL-23 and 10FS (IC$_{50}$ = 5.05 and 10.95μg/ml
respectively). The water extract of all the plants exhibited no activity against all types of human cells (Saetung et al., 2005).

Flavonoid rich extracts from the mature roots of Scutellaria baicalensis have been shown to exhibited antiproliferative effects on various cancer cell lines. The ability of an ethanolic extract of Scutellaria baicalensis root to inhibit proliferation of malignant glioma cells. Results indicated Scutellaria baicalensis not only inhibits cellular growth in recurrent and drug resistant brain tumor cell lines, but also demonstrated an increased inhibitory effect when used in conjunction with 1,3-bis (2-chloroethyl)-1-nitrosourea. The results of this study support efficacy of Scutellaria baicalensis as an anticancer agent for glioblastomas multiforme (Scheck et al., 2006).

Medicinal plants extracts of the central region of Argentina used in domestic medicine was studied for inhibition of tumor cell proliferation. The cytotoxic effect of organic extracts of the eight plant species showed cell proliferation inhibitory properties on a cell line from human breast cancer, detecting very lower values than control in extracts obtained with different solvents. The extracts of Thelesperma megapotamicum, Oxalis erythrorhiza, and Larrea divaricata showed high inhibitory activity on MCF-7 cell line proliferation (Bongiovanni et al., 2006).

To evaluated antitumor activity of sesquiterpene lactones (scabertopin (ES-2), isoscabertopin (ES-3), deoxylephantopin (ES-4), isodeoxylephantopin (ES-5) isolated from Elephantopus scaber (Linn.) in vitro and in vivo. It have been shown that (ES-2), (ES-4), (ES-5) exhibited significant antitumor effect in vitro in a concentration dependent manner. However, the effect of (ES-3) on the growth of tested cell lines was relatively weak. In HeLa cells exposed to (ES-4) for 48h, morphological changes and DNA ladder pattern evidencing on apoptosis was detected. (ES-4) revealed in vivo antitumor activity. Antitumor activity of studied sesquiterpene lactones may be due, at least in part, to induction of apoptosis in vitro. (ES-4) possesses also antitumor activity in vivo (Xu et al., 2006).

Screening of different extracts and fractions from the plant Bidens pilosa (Linn.) var. (Asteraceae) were collected from Kerala, South India and conducted using the in vitro comet assay for anticancer, which was done with in vivo models. The extract from whole plant was extracted with n-hexane, chloroform and methanol extract (E1-E3). The
extracts were fractioned by column chromatography method and fractioned with ethyl acetate, acetone and water (F1-F3). All the extracts and fractions were tested for anticancer. Among extracts E1 showed remarkable anticancer activity and E3 bears maximum antipyretic activity. The most promising material (LC₅₀ < 1500µg/ml) was F1 ethylacetate fractions of methanolic extract and methanolic crude extract of whole plants. The extract obtained from the whole plant of *Bidens pilosa* showed significant cytotoxic effect to methanolic extract against human cervix cancer cell line (HeLa) cells by *in vitro* method (Sundararajan et al., 2006).

Some of the cycloartane triterpenoids isolated from the aerial part of *Cimicifuga dahurica* showed cytotoxicity in several cancer cell lines. The total glycosides from the aerial part (TGA) was extracted and its cytotoxicity was evaluated in human hepatocellular liver carcinoma (HepG-2) cells and primary cultured normal mouse hepatocytes by an MTT assay. Morphology observation, Annexin V-FITC/PI staining, cell cycle analysis and western blot was used to further elucidate cytotoxic mechanism of TGA. Implanted mouse H₂₂ hepatoma model was used to demonstrated tumor growth inhibitory activity of TGA *in vivo*. The (IC₅₀) values of TGA in HepG-2 and primary cultured normal mouse hepatocytes were 21 and 105µg/ml, respectively. TGA induced G₀/G₁ cell cycle arrest at lower concentration 25µg/ml, and triggered G₂/M arrest and apoptosis at higher concentrations 50 and 100µg/ml respectively. An increase in the ratio of Bax/Bcl-2 was implicated in TGA-induced apoptosis. In addition, TGA inhibited growth of the implanted mouse H₂₂ tumor in a dose dependent manner. TGA may potentially find use as a new therapy for the treatment of hepatoma (Tian et al., 2007).

The polyphenolic extract (PPE) of leaves of *Ichnocarpus frutescens* was evaluated for antitumor activity *in vivo*. Results of *in vivo* study showed significant decrease in tumor volume, viable tumor cell count and a significant increase of life span in the polyphenolic extract treated group compared to untreated one: the life span of polyphenolic extract treated animals increased by 53.41% (50mg PPE/kg) and 73.95% (100mg PPE/kg). The polyphenolic extract 5, 10 and 20µg/ml effectively inhibits *in vitro* proliferation of U-937 and K-562 cell lines (Kumarappan and Mandal, 2007).

The antitumor activity of 20% methanol extract of *Urtica pilulifera* was evaluated against ehrlich ascites carcinoma (EAC) tumor model in mice. The activity was assessed
using hematological studies, solid tumor mass and survival time. Intraperitoneal injection of *Urtica pilulifera* crude extract with different doses increased survival time. Hematological parameters and packed cell volume, which were altered by tumor inoculation, were restored. Solid tumor mass was also significantly reduced. The bioactive compounds which produced these activities were isolated and identified as flavonoids and phenolic acids (Abdel-Kader et al., 2007).

The compound recipe Radix *Sophorae flavescentis* for injection is a freeze dried powder prepared from an extract of *Sophora flavescentis* and *Smilacis rhizoma*. This compound recipe Radix *Sophorae flavescentis* for injection was used to investigated antitumor effect *in vitro* and *in vivo*. MTT assay was used to examine its effect on human cervix cancer cell line (HeLa) and human hepatocellular liver carcinoma (HepG-2) cells. The compound recipe Radix *Sophorae flavescentis* for injection (dose: 1.6, 3.2 and 6.4gm/ml) produced clear inhibitory effect on the growth of S180, H22, Lewis in tumor-bearing mice, and the inhibition rate was more than 35% (P<0.01 vs control group). The compound recipe Radix *Sophorae flavescentis* for injection at a dose of 1-0.25gm/ml significantly inhibited growth of HeLa and HepG-2 cells in a dose dependent manner. It appears compound recipe Radix *Sophorae flavescentis* for injection have marked inhibitory effects on tumor growth *in vitro* and *in vivo* (Zheng et al., 2007).

The methanolic extracts of *Indigofera aspalathoides* (MEIA) and *Wedelia calendulaceae* (MEWC) was evaluated for their anticancer activity against ehrlich ascites carcinoma (EAC) in swiss albino mice. The anticancer activity of *Indigofera aspalathoides* and *Wedelia calendulaceae* was examined by determining tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span in experimental animal models. Both these extracts increased life span of EAC treated mice and restored hematological parameters as compared with EAC bearing mice. Thus, *Indigofera aspalathoides* and *Wedelia calendulaceae* showed anticancer activity in the tested animal models (Gupta et al., 2007).

Twenty one plant extracts of different species used by Indian traditional healers for the treatment of ulcers, cancers, tumors, warts, and other diseases, were tested *in vitro* for their potential anticancer (antiproliferative and cytotoxic) activity. The ethanolic extracts were tested against six human cancer cell lines using MTT 3-(4,5-dimethylthiazol-
2-yl)-2,5-diphenyltetrazolium bromide. Seven out of the 21 extracts 33\% showed remarkable cytotoxic potential. The highest activity was found in the leaf/stem ethanol extracts from *Plectranthus urticoides* and *Garcinia morella* against all the six human cancer cell lines screened (Garg et al., 2007).

The antitumor effects of peptide extracts from plants on slowly growing mammary adenocarcinoma in CBRB-Rb(8.17)11em mice used as a model of breast cancer in humans. The antitumor effect of a single injection of the test peptides was evaluated by the delay of the appearance and growth of palpable breast cancer in mice over 4 weeks. Peptides from *Hypericum perforatum* and a mixture of *Chelidonium majus* (Linn.), *Inula helenium* (Linn.), *Equisetum arvense* (Linn.), and *Inonotus obliquus* exhibited maximum activity. Peptide extracts from *Frangula alnuc* Mill. and *Laurus nobilis* (Linn.) was less active. No antitumor effect of *Camelia sinesis* Kuntze was detected (Tepkeeva et al., 2008).

Nine plants available in the Eastern Cape Province of South Africa were tested for antithrombotic and/or anticoagulant and anticancer activity. Organic (methanol) and aqueous (distilled water) extractions were performed on the various plant parts. The tannin free aqueous extract from *Leonotis leonurus* root was the only extract that affected CaCl\_2-induced clotting time. In contrast the tannin free organic extract was active against all the cell lines tested. The most cytotoxic effect was noted against HL-60 cell lines followed by the K-562 cells, and the lowest cytotoxic effect was observed against the HT-29 cell line (Kee et al., 2008).

The antitumour activities of endophytic actinomycetes isolated from pharmaceutical plants in rainforest in Yunnan Province, China. Antitumour activity was studied by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The high bioactive endophytic isolates were identified and further investigated for the presence of polyketide synthases (PKS-I, PKS-II) and nonribosomal peptide synthetases (NRPS) sequences by specific amplification. The molecular identification confirmed 41 isolates showed significant activities were members of the genus *Streptomyces*. Among them, 31.7\% of endophytic streptomycete cultures were cytotoxic against A-549 cells, 29.3\% against HL-60 cells, 85.4\% against BEL-7404 cells, 90.2\% against P388D1 cells (Li et al., 2008).
The antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts (ethyl acetate, acetone, chloroform and water) was investigated. Among the four extracts tested, the 1:8 dilution of the acetone extract was non toxic to normal cells and also have both anticancer and antiproliferative activities against cancerous cells. It confirms antitumor activities of *Stevia rebaudiana* leaves extracted using various solvents (Jayaraman et al., 2008).

1-(2-Ethyl, 6-Heptyl) Phenol (EHP), a biologically active compound formerly extracted by benzene from *Cuminum cyminum* (Cumin) Egyptian seeds exhibited antitumor activity against six types of tumor cell lines (HEPG2, HELA, HCT116, MCF-7, HEP2, CACO2) (Mekawey et al., 2009).

The ethylacetate fraction of the wampee (*Clausena lansium*) peel extract exhibited strong anticancer activities against human gastric carcinoma (SGC-7901), human hepatocellular liver carcinoma (HepG-2) and human lung adenocarcinoma (A-549) cancer cell lines, higher than cisplatin, a conventional anticancer drug. Thus, wampee peel can be used potentially as a readily accessible source of anticancer (Prasad et al., 2009).

The anticancer cytotoxic activities of isolated saponins, gymnemagenol (C$_{30}$H$_{50}$O$_{4}$) from *Gymnema sylvestre* and dasyscyphin C (C$_{28}$H$_{40}$O$_{8}$) from *Eclipta prostrata* leaves were tested under *in vitro* conditions in HeLa cells. The gymnemagenol and dasyscyphin C at 50µg/ml showed good cytotoxic activity 63% and 52%, respectively in HeLa cells at 48 hours with (IC$_{50}$) value of 37 and 50µg/ml, respectively. 5-Fluorouracil (5-FU), a positive control, showed 57.5% cell death with (IC$_{50}$) value of 36µg/ml. The percentage of HeLa cell death was maximum 73% after 96 hours with gymnemagenol, whereas dasyscyphin C showed only 53%. It can be concluded saponins, gymnemagenol, and dasyscyphin C have significant anticancer cytotoxic activity on human cervix cancer cell line (HeLa) cells under *in vitro* conditions (Khanna and Kannabiran, 2009).

*Oldenlandia diffusa* (Wild.) Roxb. a well known medicinal plant in Bangladesh have been identified for antitumor properties through *Agrobacterium tumefaciens* infection using potato disc bioassay and phytotoxic effects on radish root and seed. Significant tumor inhibition was observed at 100ppm and 1000ppm of leaf methanol extract. Maximum tumor inhibition 40.98%, 41.93% and 41.89% was observed at
1000ppm for the accessions of *Agrobacterium tumefaciens*, AtTa0112, AtAc0114 and AtSi0105, respectively (Islam et al., 2009).

The antitumor activity of *Symplocos racemosa* Roxb. (Symplococaceae) plant extracts. Chloroform, butanol and ethylacetate extract were prepared and their cytotoxic activity was determined using the XTT salt based cytotoxicity assay in 96-microplate format against one leukaemia and one cervical cancer cell line. Cyclophosphamid was used as positive control. Total 3 extracts evaluated, butanolic and ethylacetate extracts demonstrated strong cytotoxic potential overall. Cell proliferation assay showed dose dependent inhibition of cell growth. Butanolic extract was found cytotoxic against HL-60 (human leukemia cell line, IC\textsubscript{50} = 27183ng/ml), HeLa (human cervix cancer cell line, IC\textsubscript{50} = 22861ng/ml), ethylacetate extract was found to be less cytotoxic against HL-60 (IC\textsubscript{50} = 117084ng/ml), HeLa (IC\textsubscript{50} = 137151ng/ml). Chloroform extract displayed no cytotoxicity against both cell lines (Bhuvan et al., 2009).

*Ageratum conyzoides* have been used in folklore for the treatment of a wide range of diseases in Nigeria. *In vitro* activity of ethanol, petroleum ether, ethylacetate, butanol, and water extracts of *Ageratum conyzoides* were screened in some cancer cell lines using the sulforhodamine B (SRB) assay. These cell lines include: human non-small cell lung carcinoma (A-549), human colon adenocarcinoma (HT-29), human gastric carcinoma (SGC-7901), human golima (U-251), human breast carcinoma (MDA-MB-231), human prostate carcinoma (DU-145), human hepatic carcinoma (BEL-7402), and mouse leukemia (P-388) cancer cell lines. The results showed ethylacetate extract exhibited highest cytotoxic activity on A-549 and P-388 cancer cells with (IC\textsubscript{50}) values of 0.68 and 0.0003µg/ml, respectively (Adebayo et al., 2010).

The leaves of *Strobilanthes crispus* which is native to the regions of Malaysia was used in folk medicine for their antidiabetic, diuretic, anticancer and blood pressure lowering properties. Crude extracts of this plant have been found to be cytotoxic to human cancer cell lines and protective against chemically induced hepatocarcinogenesis in rats. The cytotoxicity of various sub fractions of dichloromethane extract isolated from the leaves of *Strobilanthes crispus* was determined and the anticancer activity of one of the bioactive sub fractions, SC/D-F9, was further analysed against in breast and prostate cancer cell lines (Yaacob et al., 2010).
The antitumour activity of the ethanolic extract of leaves of *Holoptelea integrifolia* (EHI) have been evaluated against dalton's ascitic lymphoma (DAL) in swiss albino mice at the dose of (250 and 500mg/kg b.wt.). The experimental parameters was evaluated like tumour volume, tumour cell count, viable tumour cell count, mean survival time and increase in life span to assess antitumour activity. The extract were administered orally for 14 consecutive days to tumour bearing group of animals. The extract increased life span of DAL treated mice and restored hematological parameters as compared with DAL bearing mice in a dose-dependant manner. *Holoptelea integrifolia* showed significant antitumour activity in tested animal models (Lakshmi et al., 2010).

*Rubia cordifolia* a traditional ayurvedic medicinal plant were collected from Mumbai, India tested for antitumor activity. Dichloromethane fraction of *Rubia cordifolia* extract exhibited potent inhibition of human leukaemia cell line and human histolytic lymphoma cell line while was found to be lesser active against normal human kidney cells displaying safety for normal cells (Patel et al., 2010).