Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the developed and developing countries, as the people are becoming aware of the potency and side effects of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Throughout the history of mankind, many infectious diseases have been treated with herbals. Herbal preparations called "Phytopharmaceuticals" are preparations made from different parts of plants. They come in different formulations and dosage forms including tablets, capsules, powder, extract, tincture and cream (Samantha et al., 2000). The misuse of herbal medicine or natural products starts with wrong identification. Hence, standardization of herbal raw material is very important today before subjecting the plant material to biological screening.

Pharmacognostical studies

Pharmacognostic study is the initial step to confirm the identity and assess the quality and purity of the crude drug. Pharmacognostic techniques used in plant standardization include macroscopical, microscopical and physicochemical parameters (Pramod and Jayaraj, 2011; Ravichandra and Parakh, 2011). According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (Anonymous, 2002).

It is globally accepted that herbal based drugs have many advantages over the synthetic drugs. However, one of the major problems in utilization of phytodrugs is correct diagnosis of the medicinal plants that are used either in the traditional systems.
or modern systems of preparation of the drugs. It is regrettable to note that most of the people involved in the manufacture or preparation of herbal drugs lack the basic background of botanical knowledge of the drugs. Consequently adulteration or substitutions of plants in the place of original ones permeate the pharmaceutical industries, rendering the herbal drugs undependable and invalid. This will lead unpopularity of phytodrugs among the people. So, it is most essential that a medicinal plant, when found to be of high pharmacological potentials, should be subjected to thorough botanical standardization so that these wouldn’t any ambiguity with respect to botanical identity of the plants. Identifications of plants involve the study of the external features of vegetative and floral parts. This study must be complimented with anatomical parameters which are very often useful to identify the fragmentary plant specimens. Raw drugs pose problem of identifications and to establish their genuineness when they lack any external diagnostic features or organoleptic clues. During such situation, the microscopic analyses of the specimen will offer a helping hand to establish to identify of the phytodrugs. The anatomical features of the plant that are reliable for diagnosis and that are least changed due to environmental stresses include:

- Structure of the midrib
- Structure of lamina and its epidermal outgrowth.
- Surface view of foliar epidermis - epidermal cells and stomatal morphology.
- Petiolar anatomy.
- Venation pattern of the lamina.
- Gross anatomy of the stem and root.
- Ergastic cell inclusion such as; starch, crystal, tannin, mucilage etc.
Literature dealing with the anatomy of *Bacolepis nervosa* is lacking. The present study may be claimed as the first comprehensive investigation of the stem and leaf of *Bacolepis nervosa*. The present investigation has laid down a set of anatomical features of stem and leaf, which can be employed for botanical diagnosis. The following are the salient features of identification of stem and leaf of *Bacolepis nervosa*.

**Salient anatomical features of *B. nervosa***

- **Young stem** consists of crushed epidermal layer and one or two superficial layers of periderm, parenchymatous cortex, distinct endodermoid layer, discrete circular masses of fibres, secondary xylem with outer and medullary phloem.

- **Old stem** is basically similar to the young stem excepting thick cylinder of secondary xylem where wide, thick walled vessels are diffusely distributed.

- **The leaf** consists of prominent adaxial cone and thick, wide abaxial midrib.

- **The vascular strand** is wide and deep cup shaped with bicollateral vascular elements.

- **The lamina is bifacet.** It consists of large vertically oblong cells with thick cuticle in the epidermal layers. The mesophyll includes narrow palisade zone and wide, many layered spongy parenchyma.
• Adaxial epidermis is apostomatic. The epidermal cells have thick and straight anticlinal walls.

• The stomata are tetracytic type.

• The venation includes wide vein islets and branched dendroid type of vein terminations.

• The powder preparation shows wide and narrow fibres, tracheids, long, narrow vessel elements with simple, horizontal end wall perforations and epidermal peeling with stomata of tetracytic type.

• Non anastomosing, non articulated long cylindrical laticifers with granular inclusions are occasionally seen in the powder.

Microscopical evaluation is the simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and detection of adulterants and substituents (Ahmad et al., 2007; Pratima and Pratima, 2011).

**Physicochemical parameters**

Physicochemical standardization is a prerequisite in quality control of herbal drugs. The efficacy of herbal drug mainly depends upon its physical and chemical properties. Therefore, the determination of physicochemical characters for the authenticity of the drug is necessary before subjected to pharmacological activities. The qualitative and quantitative analysis of major bioactive chemical components of crude drug constitute important and reliable part of quality control protocol as any change in the quality of the drug directly affects the constituents (Mukherjee et al., 2008).
Evaluation of ash and extractive values of crude drugs help in the identification and determination of its purity and quality (Kokate et al., 2010). Loss on drying of plant materials should be determined and the water content should be controlled. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The test for loss on drying determines both water and volatile matter (Anonymous, 2002). The commonly applied parameter for the detection of impurities and adulteration of drug is the estimation of ash value, which establishes the quality and the purity of drug. Ash value can also detect the nature of the material added to the drug for the purpose of adulteration. In the present study, the total ash value is more for the leaf than the stem of B. nervosa. Both the samples have more water soluble ash than acid insoluble ash. These ash values are generally considered the index of the purity as well as identity of the drug.

The extractive values in different organic solvents are based on the quantity, which are soluble in them. It makes a valuable test to check the quality of drug and any variation in the chemical constituents may cause a change in the extractive values. Thus, it helps in the determination of the adulteration and is an index of the purity of drug. The extractive values of stem and leaf of B. nervosa were determined by successive extraction in different solvents. Since, the extractive percentage of the drug has not been reported in the literature, it may be taken as an addition to the existing stock of knowledge. The variation in the extractive values may be possible due to the presence of specific compound, solubility, soil condition, atmospheric condition and water content of the same (Nasrin et al., 2008).
Fluorescence analysis

Modern methods like powder analysis and fluorescence drug analysis are very useful in standardization of plant material. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Fluorescence studies of stem powder revealed the presence of fluorescent green with 1N aqueous NaOH, 1N alcoholic NaOH, 1N HCl, Conc. HCl, Conc.H$_2$SO$_4$, 50% H$_2$SO$_4$, Conc. HNO$_3$ and benzene under UV light of shorter wavelength. The leaf powder treated with 1N aqueous NaOH, 1N alcoholic NaOH, Conc. HCl, Conc. HNO$_3$ and ethanol revealed the presence of fluorescent green under UV light of shorter wavelength. Some constituents show fluorescent green in the visible range in daylight. The ultraviolet light produces fluorescent green in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. The organic molecules absorb light over a specific range of wavelength and reemit radiations and hence it can be used for the identification of the powdered drug, extract or fractions of herbs (Rashida et al., 2012). Crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs (Zhao et al., 2011).

Preliminary phytochemical analysis

The screening of plants for medicinal value has been carried out by number of workers with the help of preliminary phytochemical analysis (Ram, 2001). Phytochemical analysis of the stem and leaf extracts of *B. nervosa* revealed the presence of phytochemicals such as alkaloid, anthraquinone, catechin, flavonoid, coumarin, phenol, quinone, saponin, steroid, glycoside, tannin, sugar, terpenoid and
xanthoprotein in them. HPTLC investigation also confirmed the presence of alkaloid, flavonoid, glycoside, saponin, steroid and terpenoid which could make the plants useful for treating different ailments and having potential of providing useful drug of human use. This is because, the pharmacological activity of any plant is usually traced to a particular compounds. These components were well known to have curative activity against several human problems such as diuretic, choleric, spasmodic, chronic eczema, diarrhea, dysentery and menstrual disorders (Brinkhaus et al., 2005).

Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have antiinflammatory effects (Orhan et al., 2007). Presence of phenols indicates the plant ability for antimicrobial activities (Parekh and Chanda, 2007a). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994). Several workers have reported the analgesic (Antherden, 1969), antispasmodic and antibacterial (Okwu, 2004) properties of alkaloids. Saponins help in controlling cholesterol and diabetes (Ong, 2004). Saponins are also responsible for central nervous system activities (Rupasinghe et al., 2003). Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001).

Flavonoids are thought to play a role in protection of plants from microbial and insect attack. Moreover, flavonoids have remarkable health promoting effects, such as antiinflammtory (Yamamoto and Gaynor, 2001), antimicrobial (Tim Cushnie and Lamb, 2005), antioxidant (Shahidi and Wanasundara, 1992), anticancer (Wei
et al., 1990) activity as well as the prevention of osteoporosis (Migliaccio and Anderson, 2003). Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism. Tannins play an important role such as potent antioxidant (Trease and Evans, 1992). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). Terpenoids exhibit various important pharmacological activities i.e., antiinflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities (Mahato and Sen, 1997). Terpenoids are very important in attracting useful mites and consume the herbivorous insects (Kappers et al., 2005). Present investigation showed that this plant is a warehouse of chemodiversity.

FTIR analysis

Spectral differences are the objective reflection of componential differences. By using the macroscopic fingerprint characters of FTIR spectrum, we can trace the constituents in plant powders, identify the medicinal materials true or false and even evaluate the qualities of medicinal materials. So, FTIR spectrum reflecting objectively the panorama of chemical constituents in complex system is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine. The FTIR analysis of stem and leaf powder of B. nervosa gave results that suggest the presence of different functional groups ranging from O-H stretching, hydroxyl (3366.78 cm⁻¹ and 3386 cm⁻¹), C-H stretching, alkyl (2925.64 cm⁻¹ and 2924.36 cm⁻¹), C=O stretching, carboxylic, carbonyl (1652.41 cm⁻¹ and 1652.08 cm⁻¹), C-O bending, alcohols, esters, carboxylic acid and anhydrides (1384.09 cm⁻¹ and 1384.07 cm⁻¹) and C-Cl stretching, chlorinated compounds.
(763.99 cm\(^{-1}\) and 764.66 cm\(^{-1}\)). Therefore the FTIR analysis of stem and leaf of *B. nervosa* displayed novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also to identify the medicinally important plants.

**GC-MS analysis**

GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid and nitro compounds (Muthulakshmi *et al.*, 2012). In the present study, 30 and 31 compounds have been identified from ethanol extract of the stem and leaf of *B. nervosa* respectively by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The GC-MS analysis revealed that the ethanol extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These phytochemicals are responsible for various pharmacological actions like antimicrobial, antioxidant, anticancer and antiinflammatory activities. Among the identified phytochemicals, thymol, eugenol, piperine, phenol 2, 5-bis (1, 1 dimethyl ethyl), n-Hexadecanoic acid and lupeol have the property of antioxidant and antiinflammatory activity as reported by earlier workers (Lalitharani *et al.*, 2009; Maruthupandian and Mohan, 2011).

In the present study, thymol was identified as major compound in stem (30.47%) and leaf extracts (28.51%) of *B. nervosa*. Thymol is a monoterpane phenol derivative of cymene. Thymol is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides such as carvacrol. In addition, thymol can reduce bacterial resistance to common drugs such as penicillin (Kavitha and Holley, 2010). Numerous studies have demonstrated that naturally, the antimicrobial effect of thymol, ranging from inducing
antibiotic susceptibility in drug resistant pathogens to powerful antioxidant properties (Undeger et al., 2009). Thymol has been shown to be an effective fungicide (Ahmad et al., 2010). Thymol was demonstrated to have a strong antimutagenic effect (Mezzoug et al., 2007). In addition, there is an evidence that thymol has antitumour properties (Andersen, 2006). Though the exact mechanism is unknown, some evidence suggests that thymol affects at least some of its biocidal properties by membrane disruption (Trombetta et al., 2005).

Eugenol comprising of 9.53% and 10.74% in the stem and leaf extracts of B. nervosa was recorded in our study. Eugenol is a member of the phenylpropanoids class of chemical compounds. Eugenol is used in perfumeries, flavourings, essential oils and in medicine as a local antiseptic and anesthetic (Jadhav et al., 2004). Eugenol kills certain human colon cancer cell lines in vitro (Jaganathan et al., 2011).

The studies on the active principles in the stem and leaf extracts of B. nervosa by GC-MS analysis clearly showed the presence of piperine (5.66% and 5.54%). Piperine is an alkaloid. It has also been used in some forms of traditional medicine and as an insecticide. Piperine has been found to inhibit human CYP3A4 and P-glycoprotein enzymes important for the metabolism and transport of xenobiotics and metabolites (Bhardwaj et al., 2002). In animal studies, piperine also inhibited other enzymes important in drug metabolism (Atal et al., 1985; Reen et al., 1993). By inhibiting drug metabolism, piperine may increase the bioavailability of various compounds and later the effectiveness of some medications. The exact metabolism of piperine’s bioavailability enhancing abilities is unknown. In February 2008, researchers discovered that piperine can stimulate pigmentation in the skin, together with the exposure to UVB light (Faas et al., 2008). Piperine has shown ‘anti-
depression like activity’ and cognitive enhancing effects in rats (Wattanathorn et al., 2008). Piperine has shown antiinflammatory and antiarthritic effects in human interleukin-1 beta- stimulated fibroblast like synoviocytes and in rat arthritis models (Bang et al., 2009).

The major phytocompounds and its biological activities obtained through GC-MS study of stem of *Bacolepis nervosa* were tabulated as below

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the compound</th>
<th>Nature of compound</th>
<th><strong>Activity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Thymol</td>
<td>Phenolic compound</td>
<td>Antimicrobial, Antiinflammatory, Antioxidant</td>
</tr>
<tr>
<td>3.</td>
<td>Eugenol</td>
<td>Phenolic compound</td>
<td>Analgesic, Anesthetic, Allergenic, Antibacterial, Anticonvulsant, Antiinflammatory, Antioxidant, Antipyretic, Antisalmonella, Antistaphylococci, Antiseptic</td>
</tr>
<tr>
<td>4.</td>
<td>Caryophyllene</td>
<td>Sesquiterpene</td>
<td>Antitumor, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide</td>
</tr>
<tr>
<td>5.</td>
<td>Phenol, 2,5-bis (1,1-dimethylethyl)-phenol</td>
<td>Phenolic compound</td>
<td>Antimicrobial, Antioxidant, Anticancer, Antiinflammatory</td>
</tr>
<tr>
<td>6.</td>
<td>Caryophyllene oxide</td>
<td>Sesquiterpene oxide</td>
<td>Antitumor, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide</td>
</tr>
<tr>
<td>7.</td>
<td>Apiol</td>
<td>Aromatic compound</td>
<td>Abortifacient, Antimicrobial</td>
</tr>
<tr>
<td>8.</td>
<td>Ar-tumerone</td>
<td>Ketone compound</td>
<td>Antimicrobial, Antiinflammatory Anticancer</td>
</tr>
<tr>
<td>9.</td>
<td>Curlone</td>
<td>Ketone compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>10.</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>Terpene alcohol</td>
<td>Antimicrobial, Antiinflammatory</td>
</tr>
<tr>
<td>11.</td>
<td>Strophanthin</td>
<td>Glycoside compound</td>
<td>Cardiac stimulant</td>
</tr>
<tr>
<td>12.</td>
<td>Widdrol</td>
<td>Sesquiterpene alcohol</td>
<td>Antimicrobial, Antiinflammatory, Anticancer</td>
</tr>
<tr>
<td>13.</td>
<td>n-Hexadecanoic acid</td>
<td>Palmitic acid</td>
<td>Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase</td>
</tr>
</tbody>
</table>
### **Source: Dr.Duke's Phytochemical and Ethnobotanical Databases**

The major phytocompounds and its biological activities obtained through GC-MS study of leaf of *Bacolepis nervosa* were tabulated as below:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the compound</th>
<th>Compound Nature</th>
<th><strong>Activity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thymol</td>
<td>Phenolic compound</td>
<td>Antiinflammatory, Antioxidant, Antimicrobial</td>
</tr>
<tr>
<td>2.</td>
<td>Eugenol</td>
<td>Phenolic compound</td>
<td>Analgesic, Anesthetic, Allergenic, Antibacterial, Anticonvulsant, Antinflammatory, Antioxidant, Antipyretic, Antisalmonella, Antistaphylococc, Antiseptic</td>
</tr>
<tr>
<td>3.</td>
<td>Benzoic acid, 4-ethoxy-, ethyl ester</td>
<td>Aromatic compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>4.</td>
<td>Caryophyllene oxide</td>
<td>Sesquiterpene oxide</td>
<td>Antitumor, Analgesic, Antibacterial, Antinflammatory, Sedative, Fungicide</td>
</tr>
<tr>
<td>5.</td>
<td>Apiol</td>
<td>Aromatic compound</td>
<td>Abortifacient, Antimicrobial</td>
</tr>
<tr>
<td></td>
<td>Substance</td>
<td>Compound Type</td>
<td>Properties</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------</td>
<td>-------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6</td>
<td>Ar-tumerone Ketone</td>
<td>Ketone compound</td>
<td>Antimicrobial, Antiinflammatory, Anticancer</td>
</tr>
<tr>
<td>7</td>
<td>Tumerone Ketone</td>
<td>Ketone compound</td>
<td>Antimicrobial, Antiinflammatory, Anticancer</td>
</tr>
<tr>
<td>8</td>
<td>Curlone Ketone</td>
<td>Ketone compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>9</td>
<td>Ethyl p-methoxycinnamate</td>
<td>Cinnamate compound</td>
<td>Antimicrobial, Antiinflammatory</td>
</tr>
<tr>
<td>10</td>
<td>Ledol Sesquiterpene alcohol</td>
<td></td>
<td>Antimicrobial, Antiinflammatory</td>
</tr>
<tr>
<td>11</td>
<td>n-Hexadecanoic acid Palmitic</td>
<td>Palmitic acid</td>
<td>Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor</td>
</tr>
<tr>
<td>12</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>Palmitic acid ester</td>
<td>Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase</td>
</tr>
<tr>
<td>13</td>
<td>Azuleno[4,5-b]furan-2(3H)-one, 3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene)],[3aS-(3a,6a,9a,9b)]-[Eremanthin]</td>
<td>Furan compound</td>
<td>Antimicrobial, Antidiabetic, Antioxidant, Antilipidemic</td>
</tr>
<tr>
<td>14</td>
<td>Phytol Diterpene</td>
<td>Diterpene compound</td>
<td>Antimicrobial, Antiinflammatory, Anti cancer, Diuretic</td>
</tr>
<tr>
<td>15</td>
<td>9,12-Octadecadienoyl chloride, (Z,Z)-</td>
<td>Chloride compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>16</td>
<td>Oleic Acid</td>
<td>Mono unsaturated fatty acid</td>
<td>Antiinflammatory, Antiandrogenic, Cancer preventive, Dermatitisic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge, Flavor</td>
</tr>
<tr>
<td>17</td>
<td>13-Octadecenal, (Z)-</td>
<td>Aldehyde compound</td>
<td>Antimicrobial, Antiinflammatory</td>
</tr>
<tr>
<td>18</td>
<td>9,17-Octadecadienial, (Z)-</td>
<td>Aldehyde compound</td>
<td>Antimicrobial, Antiinflammatory</td>
</tr>
<tr>
<td>19</td>
<td>9,12-Octadecadienoic acid (Z,Z)-</td>
<td>Linoleic acid</td>
<td>Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarhritic Anticoronary, Insectifuge</td>
</tr>
<tr>
<td>20</td>
<td>2-Propenoic acid, 3-phenyl-, cyclohexyl ester [Cyclohexyl cinnamate]</td>
<td>Cinnamate compound</td>
<td>Antimicrobial, Antiinflammatory</td>
</tr>
<tr>
<td>21</td>
<td>9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxymethyl)ethyl ester [Glycerol 2-monooleate]</td>
<td>Ester compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td></td>
<td>Compound</td>
<td>Type</td>
<td>Antimicrobial, Antiinflammatory Anticancer, Antioxidant</td>
</tr>
<tr>
<td>---</td>
<td>---------------------</td>
<td>---------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>22.</td>
<td>Piperine</td>
<td>Alkaloid compound</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Stigmasterol</td>
<td>Steroid compound</td>
<td>Antimicrobial, Anticancer, Anti arthritic, Antiasthma, Diuretic</td>
</tr>
<tr>
<td>24.</td>
<td>Fucosterol</td>
<td>Steroid compound</td>
<td>Antimicrobial, Anticancer, Anti arthritic, Antiasthma, Diuretic</td>
</tr>
<tr>
<td>25.</td>
<td>Lupeol</td>
<td>Triterpene compound</td>
<td>Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Diuretic</td>
</tr>
</tbody>
</table>

Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study.

**Antioxidant activity**

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari and Chatterjee, 2008). 1, 1 Diphenyl-2-picrylhydrazyl (DPPH) is a kind of stable organic free radical (Eklund et al., 2005). Compared with other methods, the DPPH assay has multiple advantages such as good stability, credible sensitivity, simplicity and feasibility (Jin et al., 2006; Ozcelik et al., 2003). In this assay, the radical scavenger present in the plant extract will decolorize the purple coloured DPPH solution to yellow due to the reduction of the stable DPPH radicals to diphenyl picrylhydrazine in the presence of hydrogen donating antioxidant (Shon et al., 2003). In the present study, among the solvents tested, the ethanol extracts of stem and leaf of *B. nervosa* exhibited the highest DPPH scavenging activity. The results indicate that extracts with their proton donating ability, could serve as free radical inhibitors or scavengers acting possibly as primary antioxidants (Marxen et al., 2007).
The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hagerman et al., 1998). Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to the adjacent biomolecules (Hochstein and Atallah, 1988). In the present study, petroleum ether extract of *B. nervosa* stem and ethyl acetate extract of *B. nervosa* leaf showed maximum hydroxyl radical scavenging activity when compared to standard ascorbic acid. From the present results, it is observed that the extracts of stem and leaf have better hydroxyl radical scavenging activity as reflected in terms of percentage of inhibition.

Superoxide radical is considered a major biological source of reactive oxygen species (Alves et al., 2010). Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Meyer and Isaksen, 1995). Superoxide anions can be generated either from a membrane associated NADPH oxidase (Sabeur and Ball, 2006) or as a result of electron leakage from mitochondrial electron transport (Halliwell and Gutteridge, 2001). In the present study, ethanol extract of *B. nervosa* stem and methanol extract of leaf have shown to possess superoxide quenching ability. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of the extract towards generation of superoxide in the *in vitro* reaction mixture.
ABTS radical scavenging assay involves a method that generates a blue/green ABTS\(^+\) chromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen donating antioxidants is measured. The highest percentage of ABTS radical cation activity was found in the ethanol extract of \textit{B. nervosa} stem and ethyl acetate extract of leaf respectively. The results imply that the extracts of stem and leaf of \textit{B. nervosa} inhibit or scavenge the ABTS radicals since both the inhibition and scavenging properties of antioxidant towards ABTS radicals have been reported earlier (Re \textit{et al.}, 1999; Rice-Evans and Miller, 1997).

The reducing capacity of the extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidant involved in the reduction of Fe\(^{3+}\) to Fe\(^{2+}\) by donating an electron. Increasing absorbance at 700 nm indicates an increase in reducing ability (Olayinka \textit{et al.}, 2010). The antioxidants present in the extracts of \textit{B. nervosa} caused their reduction of Fe\(^{3+}\)/ferricyanide complex to the ferrous form and thus proved the reducing power. Higher reducing power was (0.588 ± 0.0270 and 436 ± 0.61 at 800 \(\mu\)g/ml) evident in methanol extract of stem and leaf of \textit{B. nervosa}. Previous reports suggested that the reducing properties have been shown to exert antioxidant action by donating of a hydrogen atom to break the free radical chain (Gordon, 1990).

In the present study, it was found that stem and leaf of \textit{B. nervosa} showed concentration dependent free radical scavenging activity and this antioxidant effect may be due to the higher content of alkaloids, flavonoids, steroids, glycosides and saponins, highly responsible secondary metabolite for antioxidant activities (Sathishkumar \textit{et al.}, 2009). Thus, the \textit{B. nervosa} stem and leaf extracts as promising
natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free radical mediated diseases.

**Anticancer activity**

Cancer is a multi-mechanistic second largest disease in the world and requires a multidimensional approach for its treatment, control and prevention. There are various types of tumors such as sarcoma, lymphoma, carcinoma and leukemia. In our study, Ehrlich ascites carcinoma was used to induce cancer cells in mice. The ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascetic form, it has been used as transplantable tumor model to investigate the antitumor effects of several substances (Segura *et al.*, 2000). Research is being carried out throughout the world to find a lead compound which can block the development of cancer in human. Nature has always been a great contributor towards this goal.

The results of the present study showed an anticancer effect of stem and leaf extracts of *B. nervosa* against EAC in Swiss albino mice. The animals treated with the ethanol extracts of stem and leaf of *B. nervosa* at the doses of 150 and 300 mg/kg inhibited the body weight, tumor volume, packed cell volume, tumor viable cell count and also reverted the haematological parameters to approximately near normal levels.

In the present study, there was a significant decrease in the body weight of drug treated groups compared to the tumor control. A highly significant (*p*<0.01) decrease was observed in groups treated with 300 mg/kg of extract. An increase in the relative organ weight of immunologically important organs like spleen and thymus in
the tumor control can be attributed to their increased activity and production of
immunocompetent cells. It may also be due to the accumulation of fluids. The
administration of the extract indicated a dose dependent decrease in the weight of
lymphoid organs which may be due to the bioactive compounds present in the extract
supporting the activity of the immune system. The reduction in weight of liver and
kidney on treatment may be the result of removal of toxic fluids by the action of the
extract (Meenakshi et al., 2013).

In EAC bearing mice, regular rapid increase in ascitic tumor volume was
observed. The ascitic fluid is the direct nutritional source for tumor cells and the faster
increase in ascitic fluid with tumor growth could possibly be a means to meet the
nutritional requirements of tumor cells (Dahanukar et al., 2000). The reliable criteria
for judging the value of any anticancer drug is the prolongation of lifespan of the
animal (Clarkson and Burchneal, 1965) and disappearance of WBC from blood
(Obeling and Guerin, 1954). Treatment with stem and leaf extracts of B. nervosa
decreased the tumor volume and increased the percentage of lifespan. It may be
concluded that stem and leaf extracts of B. nervosa, by decreasing the nutritional fluid
volume and arresting the tumor growth, thereby increased the lifespan of EAC
bearing mice.

The reduction in viable cell count and increased non viable cell count towards
normal in tumor host suggested that extracts stimulate the growth and activity of
immune cells by the production of interleukins, which target tumor cells and cause
lysis of the tumor cells by indirect cytotoxic mechanism. Furthermore, the reduced
volume of tumor and increased survival time of the mice suggest that the extract
might have exerted a delay in vascular permeability to the cells (Bhist et al., 2010).
Myelosuppression and anemia (reduced haemoglobin) have been frequently observed in ascites carcinoma (Price and Greenfield, 1950; Maseki et al., 1981). Anemia encountered in ascites carcinoma mainly due to iron deficiency either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (Fenninger and Mider, 1954). In the present study, elevated WBC count, reduced haemoglobin and RBC count were observed in EAC control mice and the oral administration of \( B. \text{nervosa} \) restored haemoglobin content and maintained normal values of RBC and WBC, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties which include cytotoxic and chemopreventive effects (Abdulaev, 2001). In the present study, vincristine was selected as anticancerous drug. The isolation of vinka alkaloids (vincristine and vinblastine) from \( \text{Catharanthus roseus} \) introduced a new era in the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005).

Thirty compounds were identified from ethanol extract of stem and leaf of \( B. \text{nervosa} \) by GC-MS analysis. Among the thirty compounds, piperine, stigmastene 3 one, phytol, widdrol, tumerone, phenol, 2, 5 bis (1,1 dimethyl ethyl), benzaldehyde 2,5 - dimethyl, lupeol and caryophyllene showed anticancer activities. There is no previous report about anticancer activity of the plant \( B. \text{nervosa} \). The outcome of present investigation undoubtedly indicates that, the treatment with \( B. \text{nervosa} \) was effective on inhibiting the tumor progression in Swiss albino mice.
Antidiabetic activity

Diabetes mellitus is a heterogeneous metabolic disorder that has affected substantial population regardless of sex, age and socio economic status (Kannur et al., 2006). The primary complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye and can result in gradual vision loss and potentially blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The prevention of diabetes is an urgent worldwide health concern. Medicinal plants could be considered as potential source for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus (Subbiah et al., 2006).

In the present study, there was a weight loss in the alloxan induced diabetic rats, whereas treatment with ethanol extracts of stem and leaf of *B. nervosa* at both the doses showed improvement in their body weight indicating that the plant extracts had beneficial effects in preventing loss of body weight of diabetic rats. The probable mechanism of this benefit is due to its effects in controlling muscle wasting (i.e.) by reversal antagonism (Whitton and Hens, 1975).

In this present investigation, effect of stem and leaf ethanol extracts of *B. nervosa* has been evaluated for its antidiabetic and antihyperlipidemic potential. Alloxan is a potent diabetogen that is reduced to dialuric acid which is then autooxidized back to alloxan resulting in the production of $\text{H}_2\text{O}_2$, $\text{O}_2$, $\text{O}_2^-$ and hydroxyl radicals and causes damages to the beta-cells of islets of langerhans.
(Vijayvargia et al., 2000). This causes a profound decrease in insulin level and consequent increase in fasting blood glucose level in diabetic control animals (Group II). Administration of test drug for 14 days (Group III, IV, V and VI) was found to regenerate the pancreatic beta-cells which results in the normal secretion of insulin. Insulin, the potent hypoglycemic hormone thereby reduces the blood glucose level significantly ($p<0.05$).

Glycosylated haemoglobin has been found to be increases over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin (Alyassin and Ibrahim, 1981). The rate of glycation is proportional to the concentration of blood glucose (Ragini et al., 2011). In the present study, the diabetic rats had shown higher levels of HbA1c compared to those in normal rats. Treatments with stem and leaf ethanol extracts of B. nervosa and glibenelamide showed a significant decrease in HbA1c levels in diabetic rats that could be due to an improvement in glycemic status.

A significant ($p<0.01$) elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats (Group II) when compared to control rats. The ethanol extracts of stem and leaf of B. nervosa were administered orally to rats for fourteen days and these extracts reversed the levels of urea and creatinine to near normal. Administration with glibenclamide also decreased the levels of urea and creatinine to some extent. It confirms the protection of vital tissues (Kidney and liver) including the pancreas, thereby reducing the causation of diabetes in the experimental animals.

The hypoglycemic activity of ethanol extract of Butea monosperma leaves was found to induce insulin release from pancreatic cells of diabetic rats
Ahmed et al. (1991) reported the ethyl acetate soluble fraction of an absolute ethanol extract of *Pterocarpus marsupium*, which significantly lowered blood sugar level with corresponding increase in insulin level in alloxan induced diabetic rats. It is evident from this study that there is an increase in insulin level in diabetic rats treated with stem and leaf extracts of *B. nervosa*. Many plants have been showed for their hypoglycemic and insulin release stimulatory effects (Ajay, 2009; Kumar et al., 2011; Ohadoma and Michael, 2011; Ravi et al., 2004; Thirumalai et al., 2011). Grover et al. (2002) have reported 45 medicinal plants and their products that have been used in the Indian traditional system of medicine and shown experimental or clinical antidiabetic activity. The most effective and commonly used antidiabetic plants are *Allium cepa, A. sativum, Aloe vera, Gymnema sylvestre, Syzygium cumini, Ficus benghalensis, Rubia cordifolia* and *Tinospora cordifolia* (Grover et al., 2002; Mohana Rao et al., 2005; Ziyyat et al., 1997).

It is very clear from the results presented in Table - 22, a significant ($p<0.05$) reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic control rats (Group - II), when compared to normal control (Group - I) and glibenclamide treated rats (Group - VII). Protein, albumin and globulin levels were found to be restored to normal with the administration of stem and leaf ethanol extracts of *B. nervosa* to the diabetic rats. These results were in accordance with the effect of *Eugenia singampattiana* and *Polygala rosmarinifolia* in diabetic rats (Kala et al., 2012 and Alagammal et al., 2012). The increased levels of serum protein, albumin and globulin in alloxan induced diabetic rats are presumed to be due to increased protein catabolism and gluconeogenesis during diabetes (Palanivel et al., 2001).
Elevation of serum biomarker enzymes such as SGOT, SGPT and ALP was observed in alloxan induced diabetic rats indicating impaired liver function, which is obviously due to hepatocellular necrosis. Treatment with ethanol extracts of *B. nervosa* and glibenclamide resulted in a decrease of transaminase activities in alloxan treated animals. In this study, it was observed that levels of ALP, SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanley *et al.*, 1999). Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities. SGOT and SGPT levels are indicators of liver function, hence restoration of normal levels indicate normal function of liver (Ghosh and Suryawanshi, 2001).

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, VLDL-C, PL and LDL/HDL in control and experimental rats were investigated in the present study. When compared to normal rats, the alloxan induced diabetic rats showed a significant (*p*<0.01) increase in serum lipid profiles except HDL-C, which was decreased in the diabetic rats than normal rats. The diabetic rats treated with ethanol extracts of stem and leaf of *B. nervosa* and glibenclamide showed a significant (*p*<0.05; *p*<0.01) decrease in the content of lipid profiles comparing to diabetic control rats. Similarly, HDL-C level increased in plant extract treated rats when compared to diabetic rats. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. A variety of derangements in metabolic and regulatory mechanisms due to insulin deficiency are responsible for the observed accumulation of lipids (Rajalingam *et al.*, 1993). Further, it has been reported that diabetic rats treated with insulin showed
normalized lipid levels (Pathak et al., 1981). Diabetic rats treated with B. nervosa stem and leaf extracts and glibenclamide also showed normalized lipid levels. Thus, the results indicate that stem and leaf extracts of B. nervosa may also possess insulin like action by virtue of the ability to lower the lipid levels. These results are similar to earlier reports observed with the other plants (Alagammal et al., 2012; Maruthupandian et al., 2010). The present study reveals that the levels of serum lipid profiles are usually raised in diabetic rats and such an elevation represents a risk factor for coronary heart diseases (Mironova et al., 2000). Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of cardiovascular disease (Scott and Grundy, 1999).

During diabetes, there is an enhanced activity of the enzyme resulting in an increased lipolysis releasing more fatty acids into the circulation (Agarth et al., 1999). The increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediator removal of LDL-cholesterol and decreased activity of insulin, during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats (Mironova et al., 2000). The increased concentration of free fatty acid may be due to lipid breakdown and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation. Phospholipids are increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core (Cohn and Roth, 1996). Increased phospholipids level in tissues was
reported by Venkateswaran et al. (2002) and Pari and Satheesh (2004) in streptozotocin induced diabetic rats. Administration with the stem and leaf ethanol extracts of *B. nervosa* and glibenclamide decreased the level of phospholipids.

The results of the present study showed increased lipid peroxidation (LPO) on serum, liver and kidney of alloxan induced diabetic rats. Earlier studies have confirmed that there is an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha and Pari, 2003a and Ananthan et al., 2004). Lipid peroxidation is a normal phenomenon involved in peroxidative loss in unsaturated lipids, thus bringing about lipid degradation and membrane disorganization. Peroxidized lipid has been considered to play a significant role in the pathogenesis of several diseases and may be taken as molecular mechanism of cell injury under pathological conditions.

In the present study, an increase in the levels of LPO was found and these levels were significantly (*p*<0.05; *p*<0.01) reduced after the supplementation with the ethanol extracts of stem and leaf of *B. nervosa* and glibenclamide. This indicates that stem and leaf extracts of *B. nervosa* inhibit oxidative damage due to the anti-oxidative effect of ingredients present in them. This could be correlated with the previous studies of Pari and Latha (2002) on *Cassia auriculata* flower, Prince and Menon (1998) and Prince et al. (2004) on *Syzigium cuminii*, Prince et al. (1999) on *Tinospora cordifolia* and Latha and Pari (2003b) on *Scoparia dulcis* indicating antiperoxidative and antihyperlipidemic effects in diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the
activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids (Loci et al., 1994).

The levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and reduced glutathione (GSH) in the serum, liver and kidney of the control and experimental rats were studied. A highly significant reduction in the activity of scavenging mitochondrial enzymes is observed in alloxan induced rats. These adverse changes could be reversed to near normal with the treatment of stem and leaf ethanol extracts of B. nervosa and glibenclamide. The results were in accordance with the effect of Polygala rosmarinifolia (Nishanthini and Mohan, 2012).

The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress, SOD scavengers the superoxide radical by converting it to hydrogen peroxide and molecular oxygen (Robinson, 1998) while CAT brings about the reduction of hydrogen peroxides and protects higher tissues from the highly reactive hydroxyl radicals (Brioukhanow and Netrusov, 2004). In the present investigation both these enzymes registered low levels of activity in diabetic controls indicating diabetes induces stress. Such a decline in these enzyme activities has been reported earlier (Shanmugasundaram et al., 2011). When stem and leaf extracts of B. nervosa administrated to the diabetic rats improved both SOD and CAT activities, reflecting the antioxidant potency of plant extract. The present study indicates the reduction in the ability of SOD, CAT, GPx and GSH in alloxan induced diabetic rats (Group II). These results reveal the protective role of this plant extracts in decreasing lipid peroxidation and by normalizing antioxidant system.
The present study revealed that *B. nervosa* stem and leaf extracts had antihyperglycemic, hypolipidemic and antioxidant agent. The bioactive components responsible for the observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in the stem and leaf extracts. In the present study, phytochemical screening reported that the presence of flavonoid in stem and leaf extracts which might be the constituents responsible for these activities.

**Hepatoprotective activity**

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward and Dally, 1999). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. Liver diseases are some of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders.

Liver damage induced by CCl₄ is commonly used model for the screening of hepatoprotective activity. The rise in serum levels of SGPT, SGOT, ALP and bilirubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages.
Carbon tetrachloride induces hepatotoxicity by metabolic activation therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic functions. CCl₄ metabolically activated by CYP₄₅₀ dependent mixed oxidase in the endoplasmic reticulum to form a trichloromethyl free radical (CCl₃), which combined with cellular lipids and protein in the presence of oxygen to induce lipid peroxidation (Sureshkumar and Mishra, 2007). Assessment of liver toxicity was done by measuring the marker enzymes such as SGPT, SGOT and ALP. Ethanol extracts of stem and leaf of B. nervosa at the doses of 150 and 300 mg/kg body weight significantly restored the elevated levels of serum marker enzymes. The normalization of serum markers by the stem and leaf extracts of B. nervosa, suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl₄ induced leakages of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

Diminution of total protein and albumin induced by CCl₄ is a further indication of liver damage (Navarro and Senior, 2006). A depression in total protein is observed due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl₄ administration (Vetriselvan et al., 2011). Albumin is a single polypeptide chain. It is synthesized in liver where it amounts to 60% of hepatic protein synthesis though less than one third of hepatocytes appear to synthesize albumins at any one time. In any form of hepatocellular damage, there is an increase in the plasma acute phase proteins and a fall in the plasma concentration of albumin (Sies, 1993). The CCl₄ induced hepatotoxicity rats treated with stem and leaf extracts of B. nervosa significantly increased serum total protein towards the respective normal value, which indicates hepatoprotective activity. Stimulation of protein
synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells (Rip et al., 1985; Tadeusz et al., 2001).

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract, there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes (Wolf et al., 1997). Administration with the stem and leaf extracts of B. nervosa significantly \((p<0.05; \ p<0.01)\) decreased the level of bilirubin and increased the level of protein suggesting that it offered protection.

\(\gamma\)-glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum \(\gamma\)-glutamyl transferase is generally elevated as a result of liver disease, since \(\gamma\)-glutamyl transferase is a hepatic microsomal enzyme. Serum \(\gamma\)-glutamyl transferase is most useful in the diagnosis of liver diseases. Change in \(\gamma\)-glutamyl transferase is parallel to those of amino transferases. The acute damage caused by CCl\(_4\) increased the \(\gamma\)-glutamyl transferase level but, the same attains the normal, after treatment with the stem and leaf of B. nervosa due to its antioxidant activity.

Antioxidants or free radical scavengers are very important in protecting the cells against any damage induced by free radicals, which are produced continuously in cells either during phagocytosis or accidentally as by-product metabolites. Each biological system has certain antioxidant defense mechanisms against the aggregations of such free radicals. The balance of prooxidant-antioxidant systems must exist in the cells, while the disturbance of antioxidant prooxidant balance causes
oxidative stress (Karan et al., 1999). The body has an effective mechanism to prevent or neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH). When the balance between ROS production and antioxidant defences is lost, oxidative stress occurs which is through a series of events deregulates the cellular functions leading to various pathological conditions (Khan and Sultana, 2009). Any compound, natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Lipid peroxidation (LPO) has been postulated to the destructive process of liver injury due to CCl₄ administration. In the present study, an elevation in the levels of end products of lipid peroxidation was observed in the liver of CCl₄ treated rats. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with stem and leaf extracts of B. nervosa significantly reversed these changes. Hence, it may be concluded that the mechanism of hepatoproduction by ethanol extracts of stem and leaf of B. nervosa, is due to their antioxidant effects.

Superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical (Kharpate et al., 2007). In the present study, it was observed that the stem and leaf extracts of B. nervosa significantly ($p<0.05; \ p<0.01$) increased the SOD activity in CCl₄ intoxicated rats thereby diminished CCl₄ induced oxidative damage.
Catalase (CAT) is widely distributed in all animal tissues and which decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Arun and Balasubramanian, 2011). Administration with stem and leaf ethanol extracts of *B. nervosa* increased the activity of CAT in CCl₄ induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from CCl₄ intoxication. Glutathione peroxide (GPx) is a seleno-enzyme, two third of which (in liver) (Zaltzber *et al.*, 1999) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxides (Bishayee *et al.*, 1995). Glutathione (GSH), extensively found in cells, protects cells from electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, muscle, lungs, colon, liver, lymphocytes and brain (Orhan *et al.*, 2007). In the present study, treatment with stem and leaf ethanol extracts of *B. nervosa* increased the activities of GPx and GSH in CCl₄ induced liver damaged rats.

**Histopathology**

Liver is the largest organ and it is the target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification (Larrey, 2003). Drug and chemical induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (Watkins and Seef, 2006). CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl₄ are largely due to generation of free radicals (Shenoy *et al.*, 2001). Carbon tetrachloride induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effect of drugs or
medicinal plant extracts, by in vivo and in vitro techniques (Kiso et al., 1983, Allis et al., 1990).

In the present study, the ethanol extracts of stem and leaf of B. nervosa provided significant protection against the toxic effect of CCl₄ on liver. Preventive action of liver damage induced by the CCl₄ has widely been used as indicator of the liver protective in general (Clausion, 1989). CCl₄ produces an experimental damage that histologically resembles viral hepatitis (James and Pickering, 1976). Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (Recnagel, 1983). The toxic metabolite CC1₃ radical is produced by microsomal oxidase system binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. In view of this, the test drug mediated reduction in levels of SGOT and SGPT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CC1₄. This effect is in agreement with the commonly accepted view that serum level of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987).

Alkaline phosphate is the prototype of these enzymes that reflects the pathological alteration in biliary flow (Ploa and Hewitt, 1989). CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The extract mediated suppression of the increased serum ALP activity with the concurrent depletion of raised bilirubin suggests the possibility of both the test drug being able to stabilize biliary dysfunction in rat liver during hepatic injury. Hence, the histological studies reveal the changes, which take place during the
damage and recovery. A similar effect has been reported due to treatment with extract of *Boussingaultia gracilis* (Lin *et al*., 1994), extract of *Cyperus rotundus* (Sureshkumar and Mishra, 2004) and extract of *Curcuma longa* (Deshpande *et al*., 2003).

In addition, the absence of necrotic lesions in liver samples of the extract treated group, suggests that the hepatoprotective action may be due to membrane stabilizing effects in hepatic cells. These findings appear similar to those of an earlier study (Sree Ramamurthy and Srinivasan, 1993) where it was reported that the pretreatment of rats with *Tephrosia purpurea* offered hepatoprotection due to a membrane stabilizing effect in hepatic cells. Silymarin, a standardized extract of *Silybum marianum* is also a potent hepatoprotective agent. It reverses hepatotoxin-induced alterations of biochemical parameters and has so far been the most thoroughly investigated of all the plant substances in prevented liver damage induced by carbon tetrachloride, D-galN and paracetamol in rat models (Bahati *et al*., 2006).

The present study thus demonstrated its hepatoprotective effect against CCl4 induced hepatotoxicity in rats. Further studies are required to elucidate the mechanism of its hepatoprotective action.

**Antifertility activity**

Fertility control is an issue of global and national public health concern. Current methods of contraception result in an unacceptable rate of unintended pregnancies. Contraceptive vaccines and inhibition of spermatogenesis and sperm motility provide a potential for non hormonal male contraceptive. Use of antifertility agent is one of the methods in controlling human population. In recent years, there has
been a concern about the use of plant products in affecting fertility of humans. India has vast resources of natural products. People have been using many of the medicinal plants for inducing abortion and permanent sterility (Dixit, 1992). A large number of herbal drugs are used to control fertilization with considerable success.

The results of the present study revealed a little change in the body weight of rats treated with the stem and leaf ethanol extracts of *B. nervosa* at doses of 150 and 300 mg/kg body weight for fourteen days. The weight of testis and other accessory sex organs was decreased significantly during the experiment. Among the accessory sex organs, a significant weight reduction was noticed in the caput and caudal epididymal segment and the weight reduction was dose dependent. Significant reduction was observed in the vas deferens (VD) (*p*<0.01), seminal vesicle (SV) (*p*<0.05) and prostrate (*p*<0.05). Reduction in the weight of testis and other accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories (Sharma and Jacob, 2001). It is known that the accessory sex organs viz., epididymis and vas deferens are androgen dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It is also known that any change in circulating androgens would affect the internal micro environment of epididymis and thereby lead to the alteration in sperm motility and metabolism (Khan and Awasthy, 2003).

In the present study, the rats treated with stem and leaf ethanol extracts of *B. nervosa* showed significant decreased sperm motility (*p*<0.001) and sperm density in caudal and caput epididymal segments. Drastic effect on the nature of the normal sperms, in the caput and cauda region was observed in treated rats with the stem and leaf ethanol extracts of *B. nervosa*. Further head and tail regions of the sperm were
affected in all the treated groups (Group II, III, IV and V). The development of normal and mature sperm is the key to optimum male fertility. Decline in sperm motility in males might have affected fertilization and implantation. Inadequate concentration, sluggish or non-motile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova (Manivannan et al., 2009; Pankajakshy and Madambath, 2009). The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary (Steinberger, 1971). FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in the Leydig cells of the testis (Kerr and Klester, 1975). Many studies on the testis of rat treated with plant extracts have also revealed the inhibitory activity on the proliferation of spermatogonia in mammals (Steinberger et al., 1964; Mancini et al., 1967; Krueger et al., 1974). Spermatogenesis is a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatozoa during their preformation (Steinberger, 1971; Kerr and Klester, 1975). The results of the present study suggest that oral administration with stem and leaf ethanol extracts of *B. nervosa* may affect the normal function of the sertoli cells.

Sex cells can occur during the reproductive phase, mitotic division of the spermatogonia or during the maturation of the spermatozoa, thereby affecting the number and quality of the sperm cells produced in the testis. Among the treated groups with the ethanol extracts of stem and leaf of *B. nervosa*, Group III and V (300 mg/kg body weight) produced a significant ($p<0.01; p<0.001$) decrease in sperm density and sperm motility. This may be due to the ability of the extract at the given
dose, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (Bowman and Rand, 1985; William, 2000). The presence of immature sperms was also observed in the experimental rats treated with ethanol extracts of stem and leaf of *B. nervosa* at the dose of 300 mg/kg body weight. This suggested that 300 mg/kg body weight dose level could affect the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the decrease in the mean total sperm count. The data generated in the present study, by and large, coincide to those already reported and studied with various plant extracts (Njar *et al.*, 1995; Raji and Bolarinwa, 1997; Parveen *et al.*, 2002). The decrease in the caudal epididymal sperm count is a clear indication that the stem and leaf extracts of *B. nervosa* can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of stem and leaf ethanol extracts of *B. nervosa* on the cellular mechanisms of spermatogenesis cannot be concluded, it is likely that, the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis may be the underlying cause.

No toxic effect was observed in the liver and kidney of the rats treated with extracts of the experimental plant. It is because the liver and kidney are neither directly involved on the development nor functioning of the male reproductive system/ reproductive organs.

The present study clearly exposed a decrease in the sperm density and sperm motility in the caudal epididymis of all the treated groups which led to the proven impairment of fertility in all the treated groups. The results also indicated that the
treatment of male rats with the stem and leaf ethanol extracts of *B. nervosa* reduced the number of impregnation of females with the treated males. In addition, the number of implantations and the number of viable foetuses were also decreased. This decrease in viable foetuses observed in this study, may be due to the decrease in sperm motility and sperm density. This may be due to the effect of the plant extracts on the enzymes involved in the oxidative phosphorylation process.

The present study revealed a decrease in the serum level of testosterone. This observation was similar to the earlier findings of Udoh and Kehinde (1999); Udoh and Ekipoyong (2001) and Udoh *et al.* (2005). The reduction in the level of testosterone, observed in this investigation, could be probably due to the decrease in the levels of LH/FSH. Leydig cells secrete testosterone by the stimulatory effect of LH (Udoh and Udoh, 2005; Udoh *et al.*, 2005). In males, the reduction of testosterone level may impair spermatogenesis and causes male infertility. In addition, Zitzmann (2008) have demonstrated that physiologic concentrations of testosterone, LH and FSH play an important role in spermatogenesis. The study also revealed a dose dependent increase in the serum estrogen level. This increase might probably be due to the conversion of testosterone to estrogen (Carr and Blackwell, 1993; Chinoy and Padman, 1996).

Treatment with the ethanol extracts of stem and leaf of *B. nervosa* was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicated structural and functional alteration in testis, epididymis and seminal vesicle. Depletion of sperm count and sperm motility in the drug treated rats suggested the alteration in sperm production in the testis and maturation in the epididymis. Changes in both sperm count and sperm motility resulted in a partial infertility. This resulted in abnormal sperm function which ultimately gave rise to complete male sterility.
Among the plant based contraceptives, inhibition of male fertility after administration with normal substances has been related to decreased sperm density (Watcho et al., 2001). For male contraception, it is not necessary to stop spermatogenesis, but it is enough to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (Dwivedi et al., 1990).

The antifertility activity of *B. nervosa* has been attributed to the action of various steroidal saponin. Saponins are important mainly because of their steroid structure. They are precursors for the hemi-synthesis of birth control pills (with progesterone and estrogens) as well as similar hormones and corticosteroid. (Crabbe, 1979). From the present study, it can be concluded that *B. nervosa* is capable to suppress male fertility without altering general metabolism. Hence, the possible male contraceptive efficacy of *B. nervosa* stem and leaf extracts cannot be ignored paving way to the smooth development for the clinicians interests in clinical trials towards emergence of a potent herbal male contraceptive.

Recently many laboratories are engaged in developing male contraceptives from plants (US National Academy of Sciences, 1992). Plant products as contraceptives will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in countries where population pressure is high. Recently extensive effects have been made to study the antifertility drugs from plants (Handlesman, 1994; Khan and Awasthy, 2003; Upadhyay et al., 1993). In the present study, dose dependent treatment of *B. nervosa* stem and leaf extracts and duration suggests marked alterations in the male reproductive organs. Further studies are needed to prove whether the alterations are reversible or permanent after cessation of treatment and for understanding the exact mechanism.
Anti inflammatory activity

Inflammation is the reaction of living tissues to injury, infection or irritation. It has now the prime focused area of scientific research because majority of human population worldwide is getting affected by inflammation related disorders.

Non - steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immuno-suppressant drugs which have been usually used in the relief of inflammatory diseases worldwide for a long time. They are associated with severe adverse side effects such as gastrointestinal bleeding and peptic ulcer (Valiollah et al., 2009). Recently, many natural medicines derived from plants and marine organisms were considered effective and safer for the treatment of various diseases including inflammation and pain (Su et al., 2011).

Carrageenan induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic (Vinegar et al., 1960). The first phase (1-2 hr) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin (Britto and Antonio, 1998; Saha et al., 2007). The carrageenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non - steroidal anti-inflammatory agents (Rao et al., 2005).

The probable mechanism of anti-inflammatory action of extract may be due to its influence on the second phase of inflammation, the cyclooxygenase pathway rather than lipoxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan (Rosa et al., 1971). There are also evidences that compounds inhibiting the carrageenan
induced oedema are effective in inhibiting the enzyme cyclooxygenase (Selvam and Jachak, 2004).

Phytochemical evaluation of the various extracts of *B. nervosa* revealed the presence of flavonoids, glycosides, saponins, steroids, tannins and polyphenols. Stem and leaf of ethanol extracts of *B. nervosa* showed significant antiinflammatory activity. This significant antiinflammatory effect may be due to the inhibition of inflammatory mediators by the alkaloids and flavonoids (Khan *et al.*, 2012), glycosides or steroids (Rosa *et al.*, 1971) present in the extract.

The maximal antiinflammatory activity was observed (82.28%) at 3rd hour in stem of *B. nervosa* when compared to leaf of the same plant. GC-MS analysis of *B. nervosa* stem revealed the presence of thymol, eugenol, caryophyllene, phenol 2, 5 bis (1, 1 dimethyl ethyl), caryophyllene oxide, tumerone, 3, 7, 11, 15, tetra methyl - 2 hexadecen-1-0, widdrol, phytol, piperine and 9, 17 octa decadienol (Z). Most of these compounds are derivatives of terpenoids, alkaloids and phenolic compounds which have the role of antiinflammatory effect. Terpenoids significantly inhibit the development of chronic joint swelling. Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factors (Changa *et al.*, 2008). Further and detailed studies are in progress for the isolation of single entity responsible for antiinflammatory activity and development of suitable formulations.

**Antibacterial activity**

The occurrence of bacterial diseases is becoming common in South Asia particularly in India, because of development of antibacterial drug resistant pathogens.
To resolve the problem and to detect alternative chemotherapeutic agents, the search for novel forms from newer sources is global challenges (Koochak et al., 2010). Among the five different extracts of B. nervosa, methanol extract showed highest antibacterial activity with 14 mm against Mycobacterium smegmatis and Staphylococcus aureus (Methillin sensitive). Similarly, Adebolu and Oladimeji (2005) reported that the stem distillation extracts of Ocimum gratissimum were highly effective against Staphylococcus aureus.

The antibacterial property of B. nervosa was probably due to the presence of eugenol. Our findings are in agreement with the results of the species done with Ocimum gratissimum (Iwalkun et al., 2003). Phytol was observed to have antibacterial activity against Staphylococcus aureus by causing damage to cell membranes; as a result there is a leakage of potassium ions from bacterial cells (Inoue et al., 2005). Phytol is one of the constituents in the present study. Some alkaloids and saponin have been found to possess antibacterial activity (Osborn, 2003) and hence in the present investigation, antibacterial activity being exhibited by the extracts may be the results of the presence of alkaloids (Piperine) present in the plant which was proved by GC-MS analysis. Proteus mirabilis causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy and it is secondary invader of ulcers and pressure sores (Cheesbrough, 2000; Parekh and Chanda, 2007b).

These investigations validate the use of stem and leaf of Bacolepis nervosa as a herbal drug and confirming their antioxidant, anticancer, antidiabetic, hepatoprotective, antifertility, antiinflammatory and antibacterial activities.