Anyone who has never made a mistake has never tried anything new.

-Albert Einstein
3.1. Plant collection and identification

Field exploration was undertaken in Shiradi Ghat, Bisle Ghat and Sigegudda of Hassan district; Gundya, Kukke subramanya of South Canara; Biligiriranganabetta, Gopalaswami betta of Chamarajanagar district; Kudremukh, Sringeri, Kigga of Chikmagalur and Agumbe of the Shimoga district of Western Ghats of Karnataka (Fig.1). The study area harbours diverse types of vegetation such as evergreen, semi-evergreen, moist, dry deciduous, and scrub forest which are rich in diversity of plants with medicinal values. During field visits contact was established with tribal people through interviews and discussions. The information about plants regarding distribution, parts of plant used for preparation of drugs and mode of administration were recorded. The ethno medicinal plants were collected from the forest areas with the help of folk practitioners and taxonomic identification was made using the floras, reference herbaria and in consultation with taxonomists. The medicinal legumes collected were photographed and voucher specimens were deposited in the Biodiversity laboratory, Department of Environmental Science, University of Mysore.
3.2. Preparation and Extraction of plant samples

The leaves of the *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides* and *Tephrosia tinctoria* were shade dried for 14 days at room temperature in a clean environment to avoid contamination and powdered in a domestic grinder. The powdered materials were stored in sterile glass bottles. Extracts of these plants was prepared by maceration and Hot Continuous Extraction (Soxhlet) method (Harvey, 2000)

3.3. Choice of solvents for extraction

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic
absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants (Parekh et al., 2006). In the present research work Hexane, Chloroform, Petroleum ether, Methanol and Water were used for the extraction of phytochemicals based on polarity of the solvents.

3.4. Maceration

The powdered leaves (300 g) of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides* and *Tephrosia tinctoria* were placed in a stoppered container in a dark condition with selected solvents individually such as Hexane, Chloroform, Petroleum ether, ethyl acetate, Methanol and Water, and allowed to stand at room temperature for a period of 4 days with frequent agitation until the soluble matter has dissolved. The extraction with the samples in each solvent was made twice to get sufficient crude drug. The mixture is then strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. The extract was first filtered through cheese cloth and then through Whatman filter paper No.1. The filtrate was evaporated to 50ml at room temperature and then in a vacuum
concentrator followed by Flash evaporator (Plate.8). The dried powder was kept in sterile glass bottles and used for different bioassays.

3.5 Hot Continuous Extraction (Soxhlet)

In this method, the finely powdered medicinal legumes is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvents such as Hexane, Petroleum ether, Chloroform, ethyl acetate, Methanol and Water in flask is heated and its vapours condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A (Plate.8). This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described maceration method, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This is economical in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale. The extract was first filtered through cheese cloth and then through Whatman filter paper No.1. The filtrate was evaporated to 50ml at room temperature and then in a vacuum concentrator. The dried powder was kept in sterile glass bottles and used for different bioassays.
3.6. Phytochemical screening of selected medicinal legumes

Phytochemical examinations were carried out for the selected extracts as per the standard methods (Harborne, 1984; Trease and Evans, 1989).

Alkaloids

About 0.2 g of the extracts was warmed with 2% H$_2$SO$_4$ for two minutes. It was filtered and few drops Dragendroff’s reagent was added. Orange red precipitate indicates the presence of alkaloids.

Tannins

Small quantity of extract was mixed with water chloride cell to obtain a thin layer. The cell was mounted and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones (Borntgers Test)

0.5 g of plant extract was taken into a dry test tube containing chloroform, shaken for 5 min and filtered. Equal volume of 10% ammonia solution was added. Pink violet or red colour in the ammonical layer indicates positive results.

Terpenoids

A volume of 5 ml of the plant extract was mixed in 2 ml of chloroform and concentrated H$_2$SO$_4$ was added to form a layer. A reddish brown coloration of the interface was formed indicating the presence of terpenoids.
**Flavonoids**

Five ml of dilute ammonia solution was added to the aqueous filtrate of the plant extract followed by the addition of concentrated H$_2$SO$_4$. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow colour disappeared on standing.

**Saponins**

About 2 g of the powdered sample was boiled in 20 ml of distilled water bath and filtered. The 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a suitable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. The formation of emulsion was observed.

**Steroids**

2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H$_2$SO$_4$. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

**Phlobatanins**

The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl. Formation of red precipitate indicated the presence of phlobatanins.
3.7. Anti-tubercular activity of selected medicinal legumes against *M. tuberculosis* H37Rv (ATCC 27294) by proportion assay

This assay measures the capability of the test compound or extract of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides* and *Tephrosia tinctoria* to kill (or inhibit) the multiplication of pathogenic *M. tuberculosis* H37Rv. The extracts were dissolved in dimethyl sulfoxide (DMSO) to make stocks (5 mg/ml). Serial dilutions from stocks were also made in DMSO. To 1.9 ml MB 7H10 agar medium (in tubes, temp. 45-50° C, with OADC supplement), 0.1 ml of compound or DMSO (negative control) or isoniazid* (positive control) was added. The contents were mixed and allowed to solidify as slants. Three-week old culture of *M. tuberculosis* H37Rv (from L-J medium slant) was harvested and its suspension (0.1 mg/ml, equivalent to approx. 10^7 bacilli/ml) was made in normal saline containing 0.05% Tween-80. 10 μl of this suspension (~ 105 bacilli) was inoculated on to each tube and incubated at 37°C for 4 weeks. The lowest concentration of an extract up to which there was no visible growth of bacilli was its minimal inhibitory concentration (MIC) (Mc Clachy JK, 1978).

3.8. Antimicrobial Bioassay of *Atylosia albicans* and *Tephrosia tinctoria* by NCCLS method

Five opportunistic fungal strains *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258 and five
pathogenic bacteria (Staphylococcus aureus ATCC 29213, methicillin-resistant S. aureus ATCC 43300 (MRS), Pseudomonas aeruginosa ATCC 27853, and Mycobacterium intracellulare ATCC 23068 and Escherichia coli ATCC 35281 were used in the in vitro evaluation of the crude Petroleum ether, ethanol, ethanol and water extracts of the medicinal legumes Atylosia albicans and Tephrosia tinctoria. Susceptibility testing was performed using a modified version of the National Committee for Clinical Laboratory Standards methods (Miski et al., 1983). The microbial inocula, excluding A. fumigatus, were prepared by diluting the sub cultured organism in its incubation broth. The A. fumigatus inoculum was prepared by gently removing the growth from a slant and transferring to 50 mL of YPD broth. Prepared test compounds/extracts were dissolved in DMSO, serially diluted using normal saline, and transferred in duplicates to 96-well microtiter plates. The microbial inoculum was added to achieve a final volume of 200 µL and final concentrations starting with 500µg/mL for crude extracts. Drug [Ciprofloxacin (Sigma, St. Louis, MO) for bacteria and amphotericin B (ICN Biomedicals, OH) for fungi] as well as growth and blank (media only) controls were added to each test plate. Except for M. intracellulare and A. fumigatus, which were inspected visually, all other organisms were read turbidimetrically at 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont) prior to and after incubation. For turbidimetrically read organisms, percent growth was calculated and plotted against concentration to afford the IC50/MIC. Minimum fungicidal or
bactericidal concentrations (MFC/MBC) were determined by removing 5 µL of each duplicate, transferring to agar, and incubating at 37°C for 24 h.

3.9. Anti-bacterial activity of selected medicinal legumes by Agar well diffusion method

The efficacy of the extracts of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides* and *Tephrosia tinctoria* was tested against bacteria, namely *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 7410, *Shigella flexneri* MTCC 731, *Staphylococcus aureus* MTCC 7443 and *Xanthomonas campestris* MTCC7908 by the agar-well diffusion method (Adeniyi et al., 2008). The bacterial cultures were obtained from IMTECH-MTCC Chandigarh, India. In this method, 24 h-old nutrient broth cultures of the test bacteria were swabbed uniformly on solidified sterile nutrient agar plates using a sterile cotton swab. Wells of 6 mm diameter were bored aseptically in the inoculated plates with the help of a gel puncher and the extracts (2.5, 5.0 and 10.0 mg/ml of 10% DMSO), standard (Gentamicin, 1 mg/ml) and control (10% DMSO) were added separately into the respectively labelled wells. The plates were incubated at 37°C for 24 h in an upright position and the zone of inhibition formed around the well was recorded. The experiment was carried out in triplicates and mean values were recorded.
3.10. Determination of minimum inhibitory concentration (MIC)

The MIC was determined for the selected medicinal legumes by modified agar well diffusion technique (Okeke et al., 2001). A two-fold serial dilution of the extracts were prepared by first reconstituting in 20% DMSO then diluting in sterile distilled water to achieve a decreasing concentration range of 50 to 0.781 mg/ml. A 100 µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculum (5 x 10⁵) of the test bacterial cells. All test plates were incubated at 37°C for 24 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

3.11. Antifungal activity of selected medicinal legumes by disc diffusion method

The antifungal activity of crude extracts of selected medicinal plants was tested against *Aspergillus flavus* and *Fusarium oxysporum* by disc diffusion method (Taylor et al., 1995). The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg ml⁻¹ concentrations of the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control and Nystatin (10 µg discG1) was used as positive control. The activity was
determined after 72 h of incubation at 28°C. The diameters of the inhibition zone were measured in mm.

3.12. Antioxidant activity of selected medicinal legumes and a diterpene isolated from the ethyl acetate extract of Kingiodendron pinnatum

3.12.1. DPPH radical scavenging activity

The scavenging activity of DPPH free radicals by different crude extracts of Atylosia albicans, Caesalpinia mimosoides, Derris scandens, Humboldtia brunonis, Kingiodendron pinnatum, Indigofera cassioides, Tephrosia tinctoria and a diterpene isolated from Kingiodendron pinnatum was determined according to the method reported by Gyamfi et al. (1999). Fifty microliters of each plant extract and pure compound in methanol, yielding 100 µg/ml in each reaction, was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) was used only as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, reading the absorbance at 517 nm. Ascorbic acid was used as control. The percent inhibition was calculated from the following equation:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100.
\]
3.12.2. Phosphomolybdate assay

Total antioxidant activity of crude extracts of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides* and *Tephrosia tinctoria* and diterpene isolated from the ethyl acetate extract of *Kingiodendron pinnatum* was evaluated by the formation of phosphomolybdenum Complex (Bernfeld *et al*., 1995). One hundred microliters of each methanolic solution of each extract and pure compound (10 μg/ml) was added to 1.9 ml of reagent solution (0.6 M H2SO4, 28 mM sodium phosphate and 4 mM ammoniummolybdate). The blank solutions contained only 2 ml of reagent solution. The absorbance was measured at 695 nm after 90 min.

3.12.3. Reducing power assay

The reducing power of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides*, *Tephrosia tinctoria* and a diterpene isolated from *Kingiodendron pinnatum* was determined according to the method previously described by (Oyaizu *et al*., 1986). Different concentrations of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides*, and *Tephrosia tinctoria* extracts and pure compound (diterpene) (100–600μl) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K2Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the
mixture, and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and FeCl$_3$ (0.5 ml. 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxy toluene (BHT) was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution.

3.12.4. Hydrogen peroxide- scavenging assay

The Hydrogen peroxide-scavenging activity of crude extracts of *Atylosia albicans*, *Caesalpinia mimosoides*, *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides*, *Tephrosia tinctoria* and the activity of diterpene compound from *Kingiodendron pinnatum* was determined by the method described by Ruchet *et al.*, (1984). Different concentrations of each extracts and pure compound were dissolved in 3.4 mL of 0.1M phosphate buffer (pH 7.4) and mixed with 600 μL of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, a separate blank sample was used for background subtraction. $\text{H}_2\text{O}_2$ scavenging activity $= 1 - \frac{A_s}{A_c} \times 100$.

3.13. Statistical analysis

All data were reported as mean ± standard deviation of three replicates. The data was statistically analysed by two way analysis of variance (ANOVA) for antioxidant activity and one way analysis of variance (ANOVA) for
antimicrobial activity, followed by Tukey’s multiple range test (Tukey, 1949) by using computerized SPSS package version 14.0. The (P < 0.05) was considered to be statistically significant.

3.14. Thin layer chromatography and Column chromatography

The crude ethyl acetate extract of the *Kingiodendron pinnatum* was reconstituted in ethyl acetate and spotted on analytical TLC (silica gel G600, 0.25 mm thickness, Merck) and the following solvent systems and ratios used as mobile phase to determine the eluent with optimum performance; After each separation, the TLC plate was exposed to iodine fumes and UV chamber. The solvent system giving the best separation and resolution was adopted for thin layer chromatography and Column chromatography. Column separation of the ethyl acetate extracts of *Kingiodendron pinnatum* was carried out with a glass column of internal diameter 80 mm and length 100 cm (Raghu chemicals, Mysore, India). Sufficient quantity of a column grade silica gel (100 - 200 mesh size) was wet-packed into the column using ethyl acetate: petroleum ether solvent system. A 40g amount of the crude extract was first dissolved in 20 ml of ethyl acetate, and passed through the column, continuously eluted with the mobile phase (ethyl acetate/ petroleum ether: 7:2.5ml ratio).
3.15. Direct bioautographic method for the detection of novel antibacterial compound

A thin layer chromatography (TLC) bioautographic agar-overlay method (Alagesaboopathi, 2011) was used to analyse most active components in the ethyl acetate crude extract of Kingiodendron *pinnatum* (as antibacterial agent). About 10 μl of ethyl acetate extract of *Kingiodendron pinnatum* was applied on pre-coated aluminium silica gel Merk plates. The plates were developed with petroleum ether and ethyl acetate (7:2.5 mL v/v). The TLC plates were run in triplicate. One of the strips was visualized under UV light to see if the separated spots were UV active. The second strip was used for bioautography assay and the third strip was used to identify spots with the various TLC reagents. Saponins were observed by the appearance of blue, violet and yellow spots using 10% vanillin ethanol solution. Individual Rf for each spot was measured. TLC bioautography was carried out using *Escherichia coli* and *Staphylococcus aureus*. The developed TLC plates were thinly overlaid with molten nutrient agar inoculated with an overnight culture of bacteria. The plates were incubated in a dark and humid chamber overnight at 37°C. Subsequently, the bioautogram was sprayed with an aqueous solution of 2, 3, 5 triphenyl tetrazolium chloride and further incubated at 37°C for 4 h. Microbial growth inhibition appeared as clear zones against a pink background. The Rf values of the spots showing inhibition were determined.
3.16. High performance thin layer chromatography (HPTLC) and Preparatory Thin layer chromatography

Chromatographic separation of ethyl acetate extract of *Kingiodendron pinnatum* was realized using HPTLC purchased from CAMAG, Switzerland. Plant extract was applied with 100 μl syringe on pre-coated silica gel 60F254 HPTLC plates (10 x 10 cm) with band length of 8 mm and track separation of 12 mm using Linomat V applying device. The chromatograph was developed in twin trough chamber using solvent system of ethyl acetate: petroleum ether 2.5:7 and UV Detectors Wavelength selection: 366 nm. The peaks, graph and spectra obtained are given in Plate and Table. 13. For preparative thin layer chromatography, slurry of 40 grams of silica gel in 84 ml of distilled water was applied to a hundred glass plates totally (20.3 cm square), with a thin-layer spreader (Research Specialties Co.) producing a gel layer of 250-micron thickness. The plates were allowed to stand for 10 minutes at room temperature and thereafter for 1 hour at 105 °C in hot air oven and then in a desiccators for 2 hours. Later ethyl acetate extract of *Kingiodendron pinnatum* was spotted on the plates (10μl each spot). Development of the plates was carried out using petroleum ether and ethyl acetate in the ration of 2.5ml: 7ml by the ascending method. Formation of the bands was seen under the UV light (wolfgang *et al.*, 1969)

3.17. High performance liquid chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) analysis was performed using different gradients of mobile phase in different run times.
Analysis of the fraction and separated compounds was performed using HPLC pumps and UV/VIS detectors of Waters company, USA, having reverse phase water guard Column: Symmetry C18 (5μm, 4.6*250mm) and Hamilton microliter syringe using an injection volume of 20 μl. The data analysis was done using Empower 2 software. Detection was made at 254 nm and 28°C. The HPLC mobile phase consisted of methanol and water. The mobile was filtered and degassed prior to use. All analyses were done on duplicate in each sample (Ceyhun et al., 2010).

3.18. Liquid chromatography-mass spectra of isolated compound (LC-MS)

Analyses of isolated diterpene from *Kingiodendron pinnatum* was performed with Agilent (Waldbronn, Germany) RR 1200 SL system (binary pump SL, diode array detector G1315C Starlight and automatic injector G1367C SL) connected to a micrOToF-Q mass spectrometer model from Bruker Daltonics (Bremen, Germany). Analyses were carried out using Zorbax Eclipse XDB-C18 columns (Agilent) with a size and granulation of 2.1 × 100 mm2 and 1.8 μm, respectively. Chromatographic separation was performed at a 0.5 ml/min flow rate using mixtures of solvents: A B (99.5% acetonitrile/0.5% water v/v) with a 3:2 split of the column effluent, so 0.2 ml/min was delivered to the ESI ion source. The elution steps were as follows: 0–5 min linear gradient from 10 to 30% of B, 5–12 min isocratic at 30% of B, 12–13 min linear gradient from 30–95% of B, and 13–15 min isocratic at 95% of B. After returning back to the initial conditions, the equilibration was achieved after 4 min. The micrOToF-Q mass spectrometer consisted of an ESI source operating
at a voltage of ±4.5 kV, nebulization with nitrogen at 1.2 bar, and dry gas flow of 8.0 l/min at a temperature of 220°C. The instrument was operated using the program MICROTOF control ver. 2.3, and data were analyzed using the Bruker data analysis ver. 4 package. The system was calibrated externally using the calibration mixture containing sodium formate clusters. Additional internal calibration was performed for every run by injection of the calibration mixture using the diverter valve during the LC separation. All calculations were done with the HPC quadratic algorithm. Such a calibration gave at least 5 ppm accuracy (Anna et al., 2011).

3.19. Fourier transforms infrared spectroscopy

FTIR spectra were recorded with a FTIR 460 plus Jasco. The powdered samples of fractioned and separated compound from *Kingiodendron pinnatum* were mixed with dried potassium bromide and prepared as pellets, scanned at room temperature (25±2 ºC) at 4000–400cm⁻¹ spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4cm⁻¹ were averaged. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra. Each sample was scanned under the same conditions with six different pellets. Special care was taken to prepare the pellets at the same thickness by taking the same amount of sample and applying the same pressure (Cakmak et al., 2006).
3.20. $^1$H Nuclear Magnetic Resonance, $^{13}$C Nuclear Magnetic Resonance and 2-Dimensional Nuclear Magnetic Resonance

For structural elucidation purposes, compound isolated were subjected to instrumental analysis. The NMR (both $^1$HNMR and $^{13}$CNMR) spectra were determined with the assistance of NMR instrumentation facility, Indian Institute of Science, Bangalore, India. Isolated compound were dried, weighed 5-10mg and dissolved in CDCl$_3$ used for NMR, Distortion less enhancement through polarization transfer (DEPT), Heteronuclear Multiple Quantum Coherence (HMQC), Heteronuclear Multiple Bond Connectivity (HMBC), and Rotating frame Overhause Effect Spectroscopy (ROESY). For NMR analysis the tubes were lowered into a probe between the poles of a magnet. The probe had a transmitter and receiver then adjusted to give the highest level of homogeneity and tubes were spun. The spectrum was then taken using instrument control. $^1$H and $^{13}$C NMR spectra of isolated diterpenes from ethyl acetate extract of Kingiodendron pinnatum were recorded on broker DRX 600 and Bruker Avance 800 instruments (Bruker , Karlsruhe, Germany) using CDCl$_3$ as solvent. Chemical shifts are reported in ppm.

3.21. $^1$HNMR

Proton magnetic resonance was widely employed. The proton NMR gives a measure of the absorptions of the different proton signals from a compound. The integer of the signal is proportional to the number of protons it presents, and the nature of the hydrogen is established by the chemical shifts. The absorption of the signal is generally proportional to the number of protons
coming into the resonance frequency of the signal with the results that the area under the absorption peak is proportional to the number of protons being detected. A nucleus in a region of high electron density experiences a chemical field proportionally weaker than those in a region of low electron density, and a higher field has to be applied to bring into resonance such nuclei are said to be shielded by the electrons. A high electrons density shields a nucleus and causes resonance to occur at relatively high field (with low delta value) likewise a low electron density causes resonance to occur at relatively low field (with high chemical shift value) and the nucleus is said to be de-shielded (Friebolin, 1998).

3.22. $^{13}$CNMR

$^{13}$C-NMR was used to determine the precise frequency at which each carbon comes into resonance and is determined not only by the applied field $\beta_0$, but also by minute differences in the magnetic environment experienced by each nucleus. These minute differences are caused largely by the variation in electrons in the neighbourhood of each nucleus, with the result that each chemically distinct carbon atom in a structure, when it happens to be a $^{13}$C, will come into resonance at a slightly different frequency from all the others. Each upward line in a $^{13}$C spectrum corresponds to one carbon atom.

3.23. Distortionless enhancement through polarization transfer (DEPT)

Distortionless enhancement through polarization transfer (DEPT) is a technique that allows a separate spectrum to be obtained for the $^{13}$C of CH$_3$, CH$_2$, and CH. So called because the impulse sequence used forces part of
the higher sensitivity associated with proton detection on to $^{13}\text{C}$, a process that enhances the $^{13}\text{C}$ signal intensity by polarization transfer from $^{1}\text{H}$ to $^{13}\text{C}$ (Friebolin, 1998).

3.24. Two-dimensional spectroscopy

Two-dimensional (2-D) spectroscopy is a more recent innovation. The spectrum contains signals dispersed according to two characteristic frequencies rather than one so that the numbers of distinct signals that can be resolved are more than in a normal 1D spectrum. In ‘resolved’ 2-D experiments, chemical shifts and hetero or homonuclear spin couplings are separated into two dimensions. The ‘correlation’ experiments meanwhile differ from resolved experiments in that they contain a mixing period during which coherence is transferred or evolves in the spin system. The result is a 2-D spectrum exhibiting connectivity’s, i.e. cross peaks between signals from coupled spins. The most common method of presenting this data is by contour plot as it is able to cope with crowded spectra and also allows easier determination of the frequency co-ordinates of peaks (Friebolin, 1998).
[Based on the results, *Kingiodendron pinnatum* was selected for separation, isolation and characterization of bio-active compound]

The phytochemical analysis carried out on these medicinal legumes are represented in the flow chart (Fig.2)
3.25. Conservation of medicinal plants for sustainable utilization

An extensive field survey was carried out in Bisle Ghat, Shiradi Ghat and Augumbe of the Western Ghats of Karnataka, India. The seeds of *Atylosia albicans*, *Caesalpinia mimosoides* *Derris scandens*, *Humboldtia brunonis*, *Kingiodendron pinnatum*, *Indigofera cassioides* and *Tephrosia tinctoria* were collected and identification is confirmed with the help of local flora and literature. The collected samples were kept viable until they arrive at the place of conservation. The germinated seeds were germinated and the seedlings are raised in polythene bags. The germplasm in the form of plants was maintaining in the field experimental site at the University of Mysore campus, Karnataka and seeds were stored at 4°C. Information on use of medicinal plants was collected and herbaria were prepared.