Leptospirosis is the world's most common zoonoses. It is caused by spirochaetes of genus *Leptospira* that affects humans and a wide range of animals including mammals, birds, amphibians and reptiles. Humans become accidental host when they come in contact with soil or water that is contaminated with animal urine. There are about 269 pathogenic *Leptospira* species found so far, responsible for causing leptospirosis like *L. hardjo*, *L. icterohaemorrhagiae*, *L. canicola*, *L. pamona*, *L. pyrogenes*, *L. inadai* etc. Leptospirosis in cattle may appear as acute, subacute or chronic forms. The economic importance of leptospirosis includes direct or indirect costs of abortion, loss of milk production and related veterinary costs and human infection. The conventional methods of diagnosis are time consuming, sometimes dangerous and fail to identify the causative species. In this study both sewage water plants and animal sources were chosen for the isolation of *Leptospira*. From sewage water plants only saprophytic bacteria were isolated where as from animals pathogenic strains were isolated. The pathogenic strains of *Leptospira* were further confirmed by molecular approaches like PCR, PCR-SSCP and genetic typing by direct sequencing.

PCR primers were designed based on outer membrane protein genes for *Leptospira*. To overcome the limitations associated with diagnosis of leptospirosis, PCR assay was developed targeting partial *LipL21*, *LipL32* and *LipL41* genes of pathogenic leptospires using in-house designed primers, with a product size of 385 bp, 532 bp and 427 bp, respectively. The assay targeting the partial sequence of *LipL21* was found to be specific for eight pathogenic leptospires out of nine leptospires tested. The PCR products were pooled and characterized by subjecting to restriction enzyme digestion using *Rsal* and *HindIII* restriction enzymes. *Rsal* digestion produced two fragments of size 253 bp and 132 bp whereas *HindIII* produced two fragments of size 214 bp and 171 bp, both of which confirmed the product size of primer P28/29 to be 385 bp. The 427 bp amplicon of *LipL41* thus amplified was subjected to RE characterization using *ClaI*, *TaqI* and *Rsal* restriction enzymes. Digestion with *ClaI* showed two fragments of sizes 269 bp and 158 bp; the product of *TaqI* digestion showed three fragments of sizes 158 bp, 143 bp and 126 bp whereas with *Rsal* digestion showed 263 bp fragment and 164 bp fragment. The restriction enzyme pattern of this amplicon confirmed it to be a *LipL41* partial gene.
of *Leptospira*. The 532 bp amplicon of *LipL32* was subjected to restriction digestion. The restriction enzyme pattern of this amplicon confirmed it to be the *LipL32* gene of *Leptospira* species. All isolates identity was confirmed by digesting with restriction enzymes. *AluI* yielded two fragments of 240 bp and 290 bp sizes, *HinfI* yielded two fragments of 160 bp and 370 bp sizes and *ClaI* yielded two fragments of 140 bp and 390 bp and the products were then cloned in pGEMT Easy vector.

PCR could detect the target bacterial gene without any ambiguity and showed good efficiency in detection of targeted species in the sample. Specificity and sensitivity of the PCR developed from the present studies is 100% and sufficient for simultaneous detection of these potentially pathogenic *Leptospira* species in clinical and environmental samples. This simple, rapid and cost-effective method can be applicable in a prediction system to prevent disease outbreak by these *Leptospira* species and can be considered as an effective tool for early diagnosis of leptospirosis.

In this study elevated SGOT and SGPT were observed in positive samples, which may be used as an adjunct to MAT in diagnosis of acute infection. Biochemical and molecular techniques were performed on 40 samples of serum taken from dogs. *Leptospira* positive samples identified by dark field microscopy and biochemical tests to correlate the biochemical SGOT and SGPT with different clinical manifestation of leptospirosis. A combined effort of clinical expertise like molecular techniques along with the confirmatory laboratory back up. PCR was a reliable and precise diagnosis of leptospirosis when compared to biochemical technique in our study. The research presented here will be helpful to improve diagnosis and control of leptospirosis in other endemic region.

Leptospirosis is an emerging disease for which culture and identification are partly unresolved. In fact, 16S rRNA-based sequencing is the most widely used PCR methodology that can detect such uncultivable pathogens. However, this assay has some limitations linked to potential problems of contamination, which hampers diagnosis. To overcome this, a simple PCR strategy involving targeting of the gene encoding the RNA polymerase β subunit (*rpoB*), a highly conserved enzyme. The sequence of the *Leptospira rpoB* gene was determined and compared with the published sequence. Our findings have significant implications for the development
of a new tool for the identification of *Leptospira*. The consistent use of PCR has improved the early diagnosis of leptospirosis but the limitation is that it cannot provide information on the infecting *Leptospira* strain which provides important epidemiological data.

Although there are methods for the rapid detection of *Leptospira*, but identification of species appears to be the main problem. PCR- Single strand confirmation polymorphism is a simple and powerful technique for identifying the sequence changes in amplifying the target DNA and PCR-SSCP technique has developed to identify the pathogenic bacteria. The selected PCR amplified products were denatured and separated by electrophoresis on a polyacrylamide gel and detected by silver staining. Analysis of PCR products from a bacterial strain demonstrated their characteristic DNA band patterns. The application of this technique for *Leptospira* disease diagnosis and the potential use of PCR-SSCP technique for the detection.

An attempt has been made to use the herbal medicine to cure leptospirosis either by direct killing or by inhibiting the growth of *Leptospira*. Methenolic and aqueous extract of whole plants of *Eclipta alba* and *Phyllanthus amarus* assessed to determine the mechanism(s) of its antileptosomal and antioxidant activity. In this study the antioxidant activity and radical scavenging activity of methanolic and aqueous extracts of selected plant materials were evaluated against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and 2,2’azinobis- (3-ethylbenzthiazoline- 6-sulfonic acid) (ABTS) activity. DPPH and ABTS free radical scavenging effect of extract was compared with standard antioxidant ascorbic acid and gallic acid and DNA damaging studies on *Leptospira* were also investigated. The antileptosomal activity of *Eclipta alba* and *P. amarus* were studied by tube dilution and micro dilution techniques and the results showed better inhibitory action against various species of *Leptospira*. The plant extracts were also found to be very effective against leptospirosis. The possible use of *E. alba* and *P. amarus* extracts to control leptospirosis are discussed. The spectral characteristics (IR, $^1$H-NMR, $^{13}$C-NMR and Mass Spectroscopy) of three biologically active fractions isolated from *P. amarus* and their efficacy against *Leptospira* were determined. The minimum inhibitory concentration (MIC) by DPPH and ABTS assay and minimum bactericidal
concentration (MBC) by tube dilution and micro dilution techniques of the three compounds for Leptospira were investigated by *in vitro* and *in vivo* methods. The important functional groups present in the three fractions were identified using spectral studies. Clinically the histopathological changes in the diseased liver and kidney of the mice were observed. This work reports a legitimacy study based on IR, $^1$H-NMR, $^{13}$C-NMR and MS analysis the compounds have great prospective in the investigation of complex matrices.

Novel class of pseudo-peptides were derived by coupling an amino acid with a heterocyclic moiety containing free amine group using suitable coupling agents. Preliminary pharmacological assays for leptospirosis were studied by test tube dilution, micro dilution technique. The compound 4c showed efficient inhibitory activity, as observed by the percent dead cells of leptospires when observed under dark field microscope. The compound 4c showed best activity at 25, 50 and 75 $\mu$g/ml, whereas other two compounds also showed activity but, it was less when compared to 4c. The synthesized compounds were characterized using spectral ($^1$H-NMR, $^{13}$C-NMR and MS) techniques. In particular, all the analyses led to the conclusion that the synthesized compound inhibiting the *Leptospira* a causal organism of leptospirosis can be used to develop anti-leptospiral drug.

- The collected of samples from different places in Karnataka, India from both sewage water and animals recorded the varied levels of leptospirosis, as it is evident from the present study.
- The PCR developed from the present study, has advantageous over other biochemical or microbiological tests for the early diagnosis of leptospirosis. The technique holds promise for screening large number of samples within a short span of time with different clinical manifestations.
- Development of PCR assay has been achieved by targeting partial sequence of LipL21, LipL32 and LipL41 gene of pathogenic leptospires using in-house designed P23/24, P28/29 and P30/31 primers, with a product size of 385 bp, 427 bp and 532 bp respectively.
- The PCR-SSCP developed from the present investigation can be used to screen large quantity of infected biological samples for the presence of *Leptospira*. It is a highly sensitive and less laborious method to detect
Summary and Conclusion

species-specific DNA fingerprints. Our results provide valuable phylogenetic information that is useful to determine the relationship in *Leptospira* species.

- We have developed a simple PCR strategy targeting the gene encoding the RNA polymerase β subunit (*rpoB*), a highly conserved gene. The sequence of the *Leptospira rpoB* gene was determined and compared with the published sequence. The result of phylogenetic tree dendrogram showed a diversity of polymorphism between *Leptospira* species of different origin.

- The present study comprises the use of traditional medicines to overcome the side effects caused by chemotherapeutics. The antioxidant, DNA damaging and antileptospiral activity of *E. alba* and *P. amarus* were studied by DPPH, ABTS, tube dilution and micro dilution techniques respectively, and the results showed better inhibitory activity against various species of *Leptospira*.

- The present study showed the uses of *E. alba* and *P. amarus* in the traditional system of medicine to treat leptospirosis.

- This study demonstrated the activity of different fractions of *P. amarus* against *Leptospira* induced mice and also were characterized by IR, NMR and MS. Among these one of the fraction F46 is a novel and very active compound and hither to not reported from *P. amarus* plant against the treatment of *Leptospira*.

- Bioactive compounds BC₁, BC₂ and BC₃ were isolated from *P. amarus* exhibited high antioxidant and anti-leptospiral activities which is evident from both *in vivo* and *in vitro* studies.

- A series of pseudo-peptides against *Leptospira* were synthesized and their chemical structures were elucidated by spectroscopic studies.