Abstract

Bovine brucellosis is a severe threat to livestock and mankind as it is a food-borne and occupational zoonosis. Rapid transmission, high morbidity, and mortality are the main features of zoonotic diseases, leading to great personal and economic losses within a short period of time. Therefore, prompt identification and characterization of Brucella species in livestock to control the spread of infection, and epidemiological data for the planning of disease control strategies is required. Total of 710 dairy samples were collected from different regions, out of which five hundred milk and blood samples from the animals with history of abortion were collected from different regions of Karnataka, India. Mysore, Chamarjanagara, Mandya, Mddur and Hassan. Two hundred ten samples were collected from the rural villages of Mandya District, Karnataka, India. Kennalu, Alpahalli, Chikkade, Chinakuralli, Hiremarali, Banangadi, Manchanahalli and Basthiahalli. All milk and blood samples were screened by presumptive tests by Milk Ring Test (MRT) and Rose Bengal Test (RBT) to detect brucella antibodies. Amongst a total of 710 milk and 710 blood samples, 4.6 % seropositivity by the Rose Bengal test and 3.4 % positive prevalence by Milk ring test for samples collected in Mysore, Chamarajnagar, Mandya, Mddur and Hassan was observed. The overall seropositivity was 3.3 % for Rose Bengal Test and 3.3 % for Milk Ring Test was recorded from the samples collected from the rural villages of Mandya district, Karnataka India.

PCR assay was carried out for gene bosp31 encoding an immunogenic outer membrane protein of 31 kDa of B. abortus, which is conserved in all Brucella spp. ABC transporter gene was also used for the designing of species specific primer, Brucella genus specific primers targeting bscp31 gene and ABCT gene showed the
apparent prevalence of 3.3 % of Brucella infection. Bruce ladder multiplex PCR assay was performed to all the brucella strains from different animal and geographical regions. The robust Bruce ladder speciation PCR can differentiate all the classical brucella species in a single step, including vaccine strains. The infected positive samples were confirmed as B. abortus by Bruce-ladder multiplex PCR. Low-stringency single specific primer polymerase chain reaction (LSSP-PCR) gene signatures and Single-strand confirmation polymorphism polymerase chain reaction (SSCP-PCR) was used to study polymorphic variations of Brucella species. The LSSP-PCR and SSCP-PCR gene signatures produced showed high genetic similarity and intraspecific similarity which are reproducible.

As an alternative to conventional antibiotics, medicinal plants are valuable resources for new agents against antibiotic-resistant strains. Hence, we evaluated the antibrucellosis activity of 16 different medicinal plants collected from the Western Ghats against B. abortus, B. melitensis, B. suis. Antibacterial assay was carried for the extracts of different medicinal plants, the potential and effective medicinal plants extract was subjected for purification by TLC and the bioactive metabolites were characterized by the GC-MS analysis. Among them Acacia nelotica, Terminalia arjuna, Eugenia jambolana and Callistemon citrinus showed significant antibrucellosis activity, comparatively Callistemon citrinus had the strong antibrucellosis activity. The crude sample was purified by TLC profiling, compounds with different retention factor were screened for antibrucellosis activity, spot D showed the significant activity and the bioactive metabolites were identified by GC-MS analysis. The bioactive compounds identified were reported for the first time and the bioactive metabolites identified exhibited as potential antibacterial activity against brucellosis and other Human pathogens.
Symptoms of brucellosis are not pathognomonic, diagnosis rely mostly on the laboratory tests. Hence, presumptive tests- Milk Ring Test and Rose Bengal test were used for screening. The species specific PCR and Multiplex PCR have advantage over presumptive tests. The SSCP- PCR, LSSP- PCR gene signatures can be used as an alternative for detection of brucellosis for screening a large number of clinical samples and identifies epidemiological diversity in developing countries. It also minimizes the drawback of cross-reactivity and only suspected mutants can be sequenced. For the first time the different medicinal plants from Western Ghats were screened for the antibrucellosis activity. The crude and TLC purified *C. citrinus* ethanolic extract exhibited strong antibrucellosis activity. The bioactive metabolites identified exhibited as potential antibacterial agents against brucellosis and other Human pathogens.