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Introduction

Leptospirosis is a bacterial zoonotic disease caused by Leptospira of spirochaetes that affects a wide range of animals including mammals, birds, amphibians reptiles and also human beings. Leptospirosis being recognized as the world's most common zoonoses. The infection is commonly transmitted to humans by allowing fresh water that has been contaminated by animal urine to come in contact with unhealed breaks in the skin, eyes or with the mucous membranes. Outside tropical areas, leptospirosis cases have a relatively distinct seasonality with most of them occurring August-September/February-March months of the year [1,2].

The family Leptospiraceae contains two genera, Leptospira and Leptonema. Based on antigenic determinants, the genus Leptospira is classified into two species, Leptospira interrogans and Leptospira biflexa,

Corporation, Tokyo, Japan). Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity[13]. e inhibitory e ect of DPPH was calculated according to the following formula:

Inhibition (%) of DPPH activity =A-B/A * 100

*Where A is Absorbance of control and B is Absorbance of Test.

50% of the radicals scavenging by test samples are regarded as IC_{50} value. Experiments were conducted in duplicates and were repeated for three times.

Free radical-scavenging ability by the use of a stable ABTS radical cation

e free radical-scavenging activity was determined by 2,2'azinobis-

decolouration. e scavenging e ects of extract increased with their concentrations to similar extents.

e hexane and chloroform extracts of E.alba and P. amarus selected plants exhibited very negligible or no antioxidant activity. Methanol and aqueous extracts of these plants showed interesting and consisting results. Hence, methanol and aqueous extracts were selected to evaluate their antileptospiral and DNA damaging studies. Among the two extracts tested for the in vitro antioxidant activity using the DPPH method, the crude methanolic and aqueous extracts of E. alba and P. amarus showed antioxidant activity with IC_{50} values. e DPPH activity of E.alba methanol, hot water and cold water extracts were found to be 75µg/ml, 130µg/ml and 150µg/ml respectively. Similarly methanol, hot water and cold water extracts of P. amarus were found to be 40 µg/ml followed by 120 µg/ml and 145µg/ml respectively shown in Table 1. e IC_{50} value for ascorbic acid was 25µg/ml. e results indicate that the antioxidant activity of the crude extract of P. amarus is almost similar to that of ascorbic acid. e antioxidant activity of E. alba was nearly the same when compared to ascorbic acid. However, the other solvent extracts from E. alba and P. amarus were found to be less active than ascorbic acid since their IC_{50} values were found to be higher shown in Table 1. Hence, the free radical scavenging activity of methanol extract exhibited promising antioxidant activity, which was further selected which for further antileptospiral activity and DNA damage studies.

In Both aqueous and methanolic extracts of these two medicinal herbs were used for comparison. IC_{50} values for ABTS activity of Eclipta alba methanolic, hot water and cold water extracts were found to be 60 µg/ml, 150 µg/ml and 125 µg/ml. whereas P. amarus methanolic, hot water and cold water extracts were found to be 35 µg/ml, 110 µg/ml and 125 µg/ml respectively. In ABTS radical scavenging assay, the methanol extracts of both plants had good activity, reference standard gallic acid showed 50% inhibition at 25µg/ml in ABTS model. Lower IC_{50} value implies higher antioxidant power shown Table 1 and 2; (Figure 1 & 2).

In both the assays methanolic and aqueous extracts of both the plants showed a better result of antioxidant, therefore the methanolic and aqueous extract of both the plants were used for further studies.

Con rmation of Leptospira species by PCR with speci c primers

Initially, PCR using rpoB primer, rpoB is the gene that encodes the subunit of bacterial RNA polymerase was standardized by carrying out reactions at various annealing temperatures ranging from 58° to 68°C on L. icterohaemorrhagiae species which showed ampli cation at 55°, 58.7° and 59.2°C (Figure 3). A single band of approximately 600bp was observed at 55°C annealing temperature. A set of PCR reactions were done by varying the template concentration from 5ng to 60ng and could amplify the speci c band at all the template concentrations (Figure 3).

e PCR reaction for all the Leptospira species was done and the ampli cation was obtained at 600 bp and the Leptospira DNA was con rmed for the DNA damaging studies (Figure 4).

Anti-leptospiral activity of Eclipta alba and Phyllanthus amarus

Inhibition (%) Inhibition (%) Hexane Chloroform Methanol Water Hexane Chloroform Methanol Water Ascor

water extracts were found to be 75 µg/ml, 130 µg/ml and 150 µg/ml respectively. Similarly *P. amarus* extract for methanol, hot water and cold water extracts were found to be 40 µg/ml followed by 120 µg/ml and 145 µg/ml respectively. The IC_{50} value for ascorbic acid was 25µg/ml. IC_{50} values for ABTS activity of *Eclipta alba* methanolic, hot water and cold water extracts were found to be 60 µg/ml, 150 µg/ml and 125 µg/ml. where as in *Phyllanthus amarus* methanolic, hot water and cold water extracts were found to be 35 µg/ml, 110 µg/ml and 125 µg/ml respectively. The free radical scavenging activity of methanolic extract was confirmed in the present investigation. However, the chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like tannins, reducing sugars and proteins present in the extract may be responsible for such activity. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity [21]. Some of these constituents have already

b esp0.5g 679(739(67r4(l) -7464(c>BDC 0 -1.233 TD [(b)-9703(es)5(p)/T1_0 1 Tf)4.154(0)9da)9s-5(96(mo5 67ws)5(

the improvement of medicinal herbs against leptospiral members to overcome the adverse reaction and also identify the presence of bioactive compounds in the E. alba and P. amarus as therapeutics.

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