SUMMARY AND CONCLUSION

The qualitative phytochemical analysis of different solvents of crude leaf extracts of E. indica and M. edule shows the presence of variety of phyto-constituents like alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins oils, saponins, steroids and tannins in most of the tested extracts. Acetone extract of E. indica and ethyl acetate extract of M. edule harbor copious amount of flavonoids, phenols and tannins.

The results of quantitative phytochemical study showed that ethyl acetate extract of M. edule possessed significantly higher amount of major phytochemicals (flavonoids (223.58 µg TAE/mg of extract), phenolics (328.42 µg GAE/mg of extract), proteins (139.59 µg/mg of extract) and tannins (178.75 µg TAE/mg of extract) except carbohydrate and vitamin C followed by acetone extract of E. indica. Maximum amount of carbohydrate (193.77 µg/mg of extract) and vitamin C (154.07 µg/mg of extract) content was detected in acetone extract E. indica.

Different solvents of crude leaf extracts of M. edule and E. indica expressed varying degree of antioxidant potential on all tested methods (DPPH, NO, OH, O$_2$• radicals) based on dose depended manner and they also had good reducing ability. Significant high antiradical potential was found in ethyl acetate extract of M. edule (DPPH IC$_{50}$ value 26.77 µg/ml, NO IC$_{50}$ value 27.04 µg/ml, OH IC$_{50}$ value 30.94 µg/ml, O$_2$• IC$_{50}$ value 50.75 µg/ml and FRAP EC$_{50}$ value 40.43 µg/ml) in all methods followed by acetone extract of E. indica with low IC$_{50}$ values (DPPH IC$_{50}$ value 70.32 µg/ml, NO IC$_{50}$ value 39.43 µg/ml, OH IC$_{50}$ value 43.91 µg/ml, O$_2$• IC$_{50}$ value 51.30 µg/ml and FRAP EC$_{50}$ value 48.69 µg/ml). Ethyl acetate extract of
M. edule exhibited remarkable scavenging ability on DPPH radicals with lowest IC$_{50}$ value (26.77 µg/ml) followed by nitric oxide radicals (IC$_{50}$ value 27.04 µg/ml).

All tested extracts of E. indica and M. edule harbor broad spectrum of antibacterial potential against most of the tested organisms. The results of antimicrobial activity clearly indicated that well in agar method was better than disc diffusion method. Majority of the tested extracts of two plants failed to inhibit the growth of tested fungal pathogens. Superior antibacterial activity was detected in ethyl acetate extract of M. edule followed by acetone extract against most of the tested pathogens. Ethyl acetate extract of M. edule showed highest growth inhibitory effect against S. pneumoniae (32 mm) with considerable MIC (62.5 µg/ml) and MBC and (250 µg/ml) value followed by S. epidermidis (29 mm) with lowest MIC (15.62 µg/ml) and MBC (31.25 µg/ml) value.

The results of larvicidal activity of various solvents of leaf extracts of E. indica and M. edule showed good to moderated toxic effect on test insects (A. aegypti and A. stephensi). The acetone extract of E. indica exhibited significant larvicidal potential against both tested mosquito species with lowest LC$_{50}$ (90.90 mg/l for A. aegypti; 41.38 mg/l for A. stephensi) values followed by ethyl acetate extract (151.26 mg/l for A. aegypti; 73.67 mg/l for A. stephensi). A. stephensi larvae was more susceptible than A. aegypti larvae to the extracts of both plants. The extracts of M. edule had weak toxic effect on both insect species when compared with the extracts of E. indica.

The crude acetone extract of E. indica and ethyl acetate extract of M. edule possessed high antioxidant, antimicrobial and larvicidal properties and were rich in
phyto-constituents (bioactive extracts) and subjected to GC-MS analysis and antiproliferative assay.

The results of GC-MS analysis revealed the presence of 22 compounds in leaf acetone extract of E. indica. Among them dotriacontane N-bicetyl (58%) was the dominant constituent followed by thujone (6.76%). Totally, 22 major compounds were detected in GC-MS analysis of leaf ethyl acetate extract of M. edule. Stearic acid (20.19%) was identified as prevailing compound followed by 1, 2, 3-Benzynetriol (11.30%).

Acetone extract of E. indica and ethyl acetate extract of M. edule exhibited significant antiproliferative effect on U-937 and HL-60 cell line in concentration dependent activity. Superior antiproliferative potential was observed in ethyl acetate extract of M. edule on U-937 cells with lowest IC<sub>50</sub> value (26.85 µg/ml). The U-937 cell line was more sensitive to the tested extracts than HL-60 cell line.

Based on the results of preliminary screening of antioxidant, antimicrobial, larvicidal potential and phytochemical analysis, the acetone extract of E. indica and ethyl acetate extract of M. edule were chosen for bioactivity guided isolation and structure elucidation of isolated bioactive compounds. According to the bioactivity guided isolation method, two compounds were isolated from acetone extract of E. indica with reference to larvicidal (compound 1) and antioxidant (compound 2) potential. Similarly, two compounds were isolated from ethyl acetate extract of M. edule with reference to antibacterial (compound 3) and antioxidant (compound 4) properties. Various chromatographic techniques were employed in the isolation of bioactive principles. The structural elucidation of isolated bioactive compounds
were carried out using various spectral analysis like, LC-MS, CHNS analysis, UV, FT-IR, \(^1\)H-NMR, \(^{13}\)C-NMR, DEPT-135, HMBC and HSQC.

According to the spectral data and previous literature, the bioactive compounds were identified. The compounds from E. indica i.e., compound 1 was thujone (terpenoid), compound 2 was fisetin (flavonol) and compounds from M. edule i.e. compound 3 was ursolic acid (triterpene) and compound 4 was rutin (flavonol). M. edule and E. indica served as source for thujone, fisetin, ursolic acid and rutin compounds. This study is the first report on the isolation of pure bioactive compounds from E. indica. Likewise, this is also the first attempt to isolate the above mentioned compounds in these two species. The antioxidant, antibacterial, larvicidal and antiproliferation potential of isolated bioactive compounds were investigated and their molecular docking studies were also carried out to understand the molecular interactions with selected target proteins.

All the isolated compounds expressed good to moderate antiradical activity in all tested method in concentration dependent manner. The fisetin exhibited significant free radical scavenging activity DPPH IC\(_{50}\) value 12.23 µg/ml, NO IC\(_{50}\) value 39.57 µg/ml, OH IC\(_{50}\) value 30.17 µg/ml, \(\text{O}_2^\cdot\) IC\(_{50}\) value 75.26 µg/ml and FRAP EC\(_{50}\) value 14.20 µg/ml on all tested radicals followed by rutin with lowest IC\(_{50}\) values DPPH IC\(_{50}\) value 32.99 µg/ml, NO IC\(_{50}\) value 57.34 µg/ml, OH IC\(_{50}\) value 17.06 µg/ml, \(\text{O}_2^\cdot\) IC\(_{50}\) value 31.85 µg/ml and FRAP EC\(_{50}\) value 17.29 µg/ml. Fisetin expressed superior quenching effect on DPPH radicals followed by reduction of Fe\(^{3+}\) in FRAP assay. Thujone showed remarkable larvicidal potential on both A. aegypti and A. stephensi larvae with significant LC\(_{50}\) values (4.23 ppm for
A. aegypti; 3.30 ppm for A. stephensi). Nil toxic effect on test insects were observed in remaining compounds.

Ursolic acid exhibited excellent broad spectrum antibacterial activity with lowest MIC and MBC concentrations on test pathogens. Among the tested bacteria, S. epidermidis was most sensitive (MIC & MBC = 1.56 µg/ml) to ursolic acid followed by S. pneumoniae. Rest of compounds showed nil growth inhibitory effect on all tested organisms. All isolated compounds showed notable antiproliferative activity on U-937 and HL-60 cell lines (in dose depended manner). Ursolic acid harbored higher antiproliferative potential with low IC$_{50}$ values followed by fisetin. Ursolic acid showed outstanding antiproliferative capacity on HL-60 cell line (IC$_{50}$=26.83 µM/ml) followed by U-937 cell line (IC$_{50}$=36.56 µM/ml).

The results of docking study of thujone with AeSCP-2 showed that it can effectively inhibit the AeSCP-2 protein (docking score = -8.2693). All the four compounds (rutin, ursolic acid, fisetin and thujone) showed good binding affinity with ATPase region of the topoisomerase II. The findings of ursolic acid docking study reveals that it possesses inhibitory effect on active site of the E. faecalis DNA gyrase B. Docking results of rutin indicates strong inhibition on the active site of VEGFR2 than the co-crystallized ligand N,2-dimethyl-6-(7-(2-morpholinoethoxy)Quinolin-4-ylloxy)benzofuran-3-carboxamide.

Based on the results of present investigation, this study concludes that E. indica and M. edule are natural source for harboring variety of phytoconstituents, antioxidants, antimicrobials, larvicidal and antiproliferative agents. The outcome of present research strengthen the ethanobotanical properties of E. indica and M. edule and encourages use of these plants in traditional medicine to treat various diseases.
The isolated compounds thujone (larvicide), fisetin (antioxidant and antiproliferative), ursolic acid (antibacterial and antiproliferative) and rutin (antioxidant and antiproliferative) might serve as alternative, highly potential, natural antioxidant, antibacterial and insecticidal agent. Moreover, the isolated compounds from M. edule and E. indica could be used as template for the development/discovery of new plant based drugs for human society.