SUMMARY

India is endowed with rich biota of medicinal and aromatic plants that two medicinal plants of ethnobotanical history were chosen for the present study of isolating bioactive metabolites. The two plants *O. spiralis* and *C. gynandra* were collected from the districts of Namakkal (Kolli hills in Eastern Ghats) and Kanchipuram (Medavakkam), respectively, that comes under the Kaveri agro-biodiversity region, one of the agro-biodiversity hotspots in India as reported by PPVFRA, India. Both the plants are well-known for their potent medicinal properties used by traditional healers for curing various ailments. The endophytic counterparts gains major importance for their coevolution with host plants in elicitation of bioactive secondary metabolites. Hence, plant samples collected in different seasons in a year were studied extensively for their endophytic occurrence resulted in isolation of *A. strictum* and *C. lunata*, commonly prevalent most recurring dominant fungal endophytes with overall relative colonization densities of 5.25% and 8% and 3.75% and 7% in *O. spiralis* and *C. gynandra* respectively. However the endophyte assemblages accrue for the presence of various hyphomycetes and celomycetes with mitosporic fungi such as *Geotrichum* sp., *Phomopsis* sp., *Colletotrichum* sp. *Cladosporium* sp., *A. alternate*, *Trichoderma*, *Nigrospora* sp., *Humicola*, etc. occurring mostly in wet periods from these plants growing in tropics. Further, the plant samples were extracted using different solvents of increasing polarity among which dichloromethane extracts revealed better TLC profile showing the qualitative presence of most secondary metabolites. Quantitative studies using UV, IR and Mass spectrometry suggested the presence of phenolics and terpenoids in the dichloromethane extracts. In addition, initial plant tissue culture studies for both plants standardized CG-BM 4 (2.0 mg/l TDZ) and OS-BM 3(1.5 mg/l NAA and 0.5 mg/l 2, 4-D) medium for *Cleome gynandra* and *Orthosiphon spiralis* showing high callusing frequency of 98.9% and 91.8% developed from 0.1% HgCl₂ surface sterilized leaf explants that gave better growth kinetics pattern in suspension cultures yielding higher biomass of 11.53 g and 11.15 g, respectively. This was followed by elicitation studies using a range of 2-10% concentrations of abiotic (methyl jasmonate) and different types of biotic elicitors (cell extract, filtered and autoclaved culture broth). The chosen endophytic cultures of *A. strictum* and *C. lunata* were subjected for preparation of biotic elicitor. 6% fungal cell extracts from *C. lunata* and *A. strictum* elicited higher amount of fresh weight of cells of about 13.11 g and 13.05 g in *C. gynandra* and *O. spiralis*, respectively, compared to other types. The metabolites extracted from these elicited plant cell cultures were profiled using
HPTLC along with wild plant dichloromethane extracts that revealed the occurrence and elicitation of two important bioactive compounds: Betulinic acid and Rosmarinic acid. These elicited cell extracts were then purified using HPLC that recovered 77% and 65% pure Betulinic and Rosmarinic acid with 2-fold increase of 15.4 and 13.1 mg/l of Betulinic acid and 11.0 and 6.6 mg/l of Rosmarinic acid from *O. spiralis* and *C. gynandra* cell extracts as compared to previous reports. The purified fractions of Betulinic acid and Rosmarinic acid exposed IC₅₀ values of 125 μg/ml and 62.5 μg/ml against MCF-7 and 250 μg/ml and 125 μg/ml against HT-29 cell lines, respectively. However, in DPPH radical scavenging activity, Rosmarinic acid were most efficient compared to Betulinic acid showing 69.58% of antioxidant activity at a minimal concentration of 40 μg/ml. Concurrent studies for apoptotic disclosed fragmented DNA patterns from treated cells makes evident the regulation of programmed cell death. Altogether, these suggest the triterpene and the phenolic acid to act in their own mechanism of affecting mitochondria directly inducing apoptosis causing the release of mitochondrial apogenic factors, activation of caspases, and DNA fragmentation while the latter induce apoptosis via the mitochondrial pathway causing cytochrome c release and procaspase-9 activation as well as mitochondrial membrane depolarization. The study reports exclusive data on seasonal recurrence of endophytes reported from *O. spiralis* and *C. gynandra*. And particularly, the research has its novel findings to report Rosmarinic acid for the first time in *C. gynandra* as well as in the identification and elicitation for production of Betulinic acid and Rosmarinic acid along with the fingerprinting of other metabolites being reported from novel source of *O. spiralis* and *C. gynandra* for the first time which can find application in industrial and therapeutic arenas as well as lead compounds for drug targets.