Review Article

**In vitro conservation of mangrove for pharmaceutical interest**

Abstract

Mangroves are halophytic species with unique morphological features, habituated in the intermediate zones of coastal area. They had better ecological community and serve as natural barriers. Since ancient times, mangrove forests are considered as the source of drugs, where tradition medicine and several healing practices were derived from the mangrove species. This potentiality was due the secretion of secondary metabolites to endure the extreme environmental conditions. In the recent years the utilization of mangroves was tremendously increased due to their variety of uses in industries and medicine. By this anthropogenic activity, severe loss and degradation of the mangrove forest area had been observed, which leads to negative ecological impact. To conserve these invaluable mangrove species, in vitro culture techniques was considered as the better practice. Our study reveals the impact of *in vitro* cultivation of mangroves and their necessity in the natural ecosystem.

Introduction

Intertidal zones of the coastal areas such as lagoons, riversides, marshes and shores are seized by the special community of species are termed as mangroves [1]. Mangrove species are woody and tall which can withstand the extreme saline condition, where other species fail to grow. Survival in the sovereign state was due to their special morphological and physiological features [2]. Whereas the evolution and growth, mangroves attain a special type of spread roots, prop roots and buttress roots to withstand in that niche. In natural ecosystem, mangroves multiply through germination, vegetative propagation, viviparity and cryptoviviparity. Reproductive biology of the mangrove species was highly adapted and influenced by the atmospheric interaction [3]. Coastal woodlands are widely habituated in the shorelines of tropical and sub-tropical regions [4]. Mangrove forest supports the marine and terrestrial ecosystems through immense primary productivity [5]. It acts as the better nursery for crab, prawn and fishes to extend their role in the marine ecosystem [6]. Through stabilizing the sediments, it prevents the coastal erosion [7]. It acts as a biological barrier against the natural calamities, such as salt water entry, cyclones and tsunamis. In southern India, mangrove belt protected several villages from the severe destruction of tsunami during 2004. High potential carbon sequestration was done by the mangrove species. Increase in the sea levels becoming a major threat to the loss of mangrove ecosystem, where there may be chance of complete eradication of mangrove ecosystems with in a century. Impact of habitat loss was vital than the nuclear wars and destruction. Significantly, the threatening rate was increased for the extinction of both floral and faunal species of mangrove habitations. Endemic species in the mangrove habitat were rich in the regions of Asia, Australia and Caribbean. Locally, people were well aware of the impact of mangrove conservation, but the destruction of the forest area is in tune due to the over utilization of direct sources of the mangroves [8]. In the coastal areas, the tremendous increase of the anthropogenic activity and destruction resulting in the loss of mangrove species severely and habitat loss through modification of mangrove habitats [9,10]. Afforestation of mangroves through seedling was the simplest method but there might be a lack in the source of seed. To satisfy the demand, mass multiplication of the mangroves and advanced techniques should be followed [11]. For the conservation of mangrove forest, one should have better knowledge about the geomorphology, ecohydrology and zonation of the mangrove species [12]. For the conservation of rare and endanger species, *in vitro* multiplication through tissue culture was followed even it was expensive, we should be able to culture and propagate throughout the year [13].

Mangrove as human’s fortune

The crucial role of satisfying both basic and economic needs of the people in the coastal region was attained through mangroves. It acts as a source of food, medicine, fire wood and provides raw material to build vehicles and constructions. Charcoal production from the mangrove species is much effective in having high caloric power, less smoke and showed slow burning properties. Several cellulose products such as fibre, cellophane, cellulose acetate were obtained from
Heritiera fomes. Dye obtained from the bark of *ceriops tagal* was considered as the better natural dyeing agent. Honey production was extremely high in the mangrove forest. Due to honey bees, positive impact of pollination was occurs in mangrove community. Coastal people utilize the fruits of *Rhizophora* *sp.* and *Sonneratia caseolaris* for the preparation of wine [14]. In the commercial production of dye and colorants, tannins from the *Rhizophora mucronata* were widely used [15]. Tribal people in Asia and Africa prefer the mangrove species as the remedy for their medicinal ailments [16]. To treat several medical ailments such as hepatitis, diabetes, leukemia, dyspepsia, anesthetic and stomach problems, mangrove species are utilized [17]. In folk medicine to cure asthmas, sores, diarrhea and wounds *Derris trifoliata* were used by the tribals in orissa [18]. At primary health care to treat bleeding and piles, the fruits of *Sonneratia apetala* were used [14]. Leaves of *ceriops decandra* were used in the preparation of tea and it had the potential of anticancer activity. In the field of medicine, mangroves act as the potential source of raw material. Most of the mangrove species might have the potential of several biological activities due to the presence of metabolites [19]. Secondary metabolites are rich in mangrove species leads to antimicrobial activity against the bacteria, cancer and free radicals [20]. *Rhizophora mucronata* and *Avicennia marina* had a potential to act against the β-cell inhibition of insulin secretion in type 2 diabetes. It is due the presence of tannins, alkaloids, flavonoids, saponins and triterpenes [21].

**Bioactive compound mangroves**

Mangrove forests act as a vital habitat for the fungal community. Through the mangilocious strain, it acts as a source of several bioactive compounds [22,23]. Actinomycetes in the mangrove community had the potential role in the production of numerous secondary metabolites [24]. Antihyperglycemic action in the pancreas is due to stimulation of flavonoids and tannins [25]. Even in the recent days the exact compound and their activity was not studied completely for all mangrove species. Some of the species in the mangrove community, such as *Abras precatorius*, *Acanthus ilicifolius*, *Alstonia macrophylla* plays a vital activity against the cancer.

Central nervous system disorders were also depressed by pneumatophore extract of *Xylocarpus moluccensis* than the bark extracts shows the presence of bioactive compounds (Sarkar et al., 2007). To study the bioinsecticidal activity, *Trogoderma granarium* is a potential insect of storage products and had a vital mortal impact against the meatholic extracts of halophytic species in *Tamarix boveana* [26]. In the mangrove associate *Suaeda maritime*, antioxidant activity was high in stem and leaves [27]. For the antioxidant and anti-nociceptive study, leaves of *Heritiera fomes* was taken and shows the better positive activity in ethanolic extract [28]. In silver nanoparticle synthesis of mangrove species, *Sonneratia apetala* shows the positive impact against several bacteria [29]. To study antibacterial, antidiarrhoeal, cardiotonic and antihyperglycemic activities, the bark, seed and stem extracts of *Xylocarpus granatum* were analysed and showed the positive results [30–32]. In the leaf extracts of *Terminalia catappa*, several activities were studied for the presence of antioxidant, antibacterial and anti-fungal activity [33]. For the better effect of antioxidant and anti-inflammatory in *Melaleuca leucadendron*, L butanol extract of leaves were used [34].

** Destruction of mangroves**

In the tropical and sub–tropical coasts, half of the mangrove community were vanished in recent years. Asia covers the total percentage about 42 % in the global mangrove distribution. One third of the mangroves were totally lost on the global scale. Globally Bangladesh coast seized the largest mangrove forest (Mathew et al., 2010). Due to brackish water aquaculture development, huge losses of mangrove species were recorded in Philippines, Myanmar and Thailand. Due to improper systematic methods, loss of mangrove ecosystem was recorded in the pichavaram mangrove forest in India. Over exploitation for raw source of the mangroves was the major issue for the habitat loss. Afforestation of the mangrove species was not effectively done so far, which leads to tremendous impact on the future generation [35].

**Conservation of mangroves:**

**In vitro conservation of mangrove:** *In vitro* culture of mangrove species was a tedious process in the propagation, where several species to be cultured for conservation of endangered and endemic species and also to utilize the secondary metabolites for socio-economic beneficial aspects. There might be a unique technique in the *in vitro* culture of varied species. Even in the recent days, we may have several difficulties to culture the successful mangrove species. In the root callus culture of *Acanthus ilicifolius*, we observed that the 3mg/L of 2,4-D had a better growth of callus biomass. Then the callus had a potential activity against the pulmonary carcinoma. To study the *in vitro* cytotoxicity effect of mangroves, root explants of a species of *Acanthus ilicifolius*, *Callophyllum inophyllum* and *Excocarrio agallocha* were taken, and cultured in the specified nutrient medium with different growth hormones, results the combination of the 2,4-D and BAP had better impact in the callus formation, subsequently the cytotoxicity studies showed the better possibility of producing best drugs against the carcinomas [36]. In some *in vitro* cultures, phenolic exudation was also a complication to culture the mangrove species, such was occurred in the micropropagation of *Excocarrio agallocha*, at that stage charcoal of about 4 g/L was used to control it. For root induction, IAA of 5.024 μmol was preferred and for shoot induction, MMS medium with 3.91 μmol of BAP and 1.34μmol of NAA was added [37]. In order to reveal the secret of bioactive compounds in the several mangrove species, callus induction was practiced in the leaf explants of *sesuvium portulasastrum*, MS medium along with 0.1–0.5 mg/L of NAA, IAA, 2,4−D, BAP and kinetin was preferred for better induction. (Ravinder singh et al., 2013). *Avicennia marina* was one of the major species in grey mangroves, in the *in vitro* culture of the grey mangrove species MS medium along with BA and NAA of 5.0 and 1.0 μmol/L shows the better outcome in eight weeks. Even the survival rate was also high in this composition [38]. In the protoplast culture of the mangrove species of *Sonneratia Alba* and *Avicennia marina* showed halophilic nature, and the comparative study was taken based on the different salt concentrations in the medium shows that the NaCl, KCl and Mg Cl₂ had a potential role in culture, while Ca Cl₂ had an inhibitory effect.
effect [39]. In the production of secondary metabolites, callus induction was preferred and so the stem explants of *Ceriops decandra* were taken and cultured in several combinations of growth regulators along with the MS medium, shows better result occurred in the combination of BAP and NAA in (0.5 mg and 2.5 mg / L). Modification of the MS medium was also preferred for the better culture of some species, in that way the callus of *Bruguiera cylindrica* shows better result in the half reduced concentration of the MS medium than the normal MS medium [40]. Suspension culture was also a conservation method, that instruct us that liquid medium of MS along with dichlorophenoxycetic acid and 2,4D of 0.1 μmol / L inhibitory effect was showed by the cytokinins in that culture of *Sonneratia alba* and *S. caseloarisis*. (Sasamoto et al., 2007). Salt stress plays a negative role in the callus culture of *Sesuvium Portualacastrum* L. In extreme level, at the level of 1000μM NaCl, it shows better protein content than the control, results more than optimum level of NaCl callus growth were retarded [41]. In the study of better medium for callus induction in *Bruguiera sexangula*, both the MS and amino acid medium shows better results in different concentration shows that nutrient requirement was unique for varied species and the NaCl concentration were also studied, results that the medium with 100mM had better growth [42]. While the different callus initiated parts shows the different effect on NaCl concentration. Callus obtained from the seedling had a better resistance to salt than the leaf originat called in *Bruguiera sexangula* [43-52].

**Conclusion**

Each and every terms of utilization in the mangrove ecosystems leads to destruction of their community, leads to several negative impacts in the ecological and environmental facets. As a better promotion and conservation of the mangrove species, *in vitro* cultivation and propagation might conserve the loss of mangroves from their natural habitat. If the callus induction of the mangrove species were successfully cultured, there is dire need for production of the secondary metabolites and their potential. By this propagation and conservation tremendous purpose of commercial and medicinal need must be satisfied. Even now there may be lack of success rate for better *in vitro* culture of mangrove species, but we hope the advanced research may move towards the conservation of mangrove through *in vitro* cultures.

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