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A Review on Micropropagational and Phytochemical Aspects of Some Medicinally Important Wetland Plants

Syamkumar T. S., S. Geethalakshmi*

Department of Biotechnology, Sree Narayana Guru College, KG Chavadi, 641105, Tamilnadu, India

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Abstract Wetlands are places where the soil is always moist. Plants that are found in areas that are always wet are called wetland plants. *Sphaeranthus indicus*, *Eclipta alba* and *Scoparia dulcis* all are wetland plants. The background of this study is to understand the different types of phytochemicals present in these three wetland plants and micropropagation methods using different types of explants. The bioactive constituents contained in these three wetland plants and micropropagation methods using various types of explants were learned from the previous articles. Phytochemicals such as sesquiterpene lactones, eudesmanolides, and flavonoids have been isolated from the plant *Sphaeranthus indicus. Eclipta alba* has yielded a diverse set of chemical compounds, including coumestans, alkaloids, thiopenes, saponins, quinines, flavonoids, polyacetylenes, triterpenes and their glycosides. The main bioactive compounds contained in the plant *Scoparia dulcis* are flavonoids, polyphenols, tannins and terpenoids. Micropropagation using different types of explants has been done very successfully for these three wetland plants. Phytochemicals mainly contained in these plants are alkaloids, terpenoids, steroids, flavonoids, coumestan. Explants used for micropropagation are leaf, shoot tip, nodal segments, apical buds, axillary buds and seeds. The main conclusion of this study is that all these plants have many medicinal properties that are yet to be discovered. All of which should be exposed to the latest technologies available in the field. However, the extent to which plant tissue culture can be used in these plants is also a possibility that should be considered.

Keywords Alkaloids, Terpenoids, Steroids, Flavonoids, Coumestan, Saponins, Quinines, IAA, IBA, NAA

1. Introduction

Natural resources include numerous useful plants for medicine. There is not enough care taken these days to preserve flora. Ancient people recognised the value of therapeutic herbs and actively protected them. This means that even the deadliest diseases have been partially conquered with the aid of therapeutic herbs. In the name of industrialisation, encroachment has had a devastating effect on the availability of therapeutic plants. Rivers, ponds, lakes, paddy fields, and other natural areas all provide usable sources of water. There is moisture in the areas surrounding them. Many useful medicinal plants can thrive in such wet conditions. Even there, the invasion is causing their resources to dwindle. Plant tissue culture offers a solution to the problem of dwindling resources, because little pieces of tissue called explants can be used to grow hundreds of thousands of plants continuously. In a short amount of time and space, regardless of the season or weather, a single explant can be grown into thousands of plants under controlled conditions [29].

2. Micropropagational Aspects of Some Wet Land Plants

An important stage in micropropagation is to select a healthy explant. Explants used for micropropagation include nodal segments, apical meristems, roots, cotyledons, embryo, leaf-disc, leaf-blade, pedicle, petiole, anther, ovary and others.

2.1. Leaf as Explant

Sphaeranthus indicus leaves were grown in isolation and then cultured with kinetin (1.3, 2.3, 4.6, and 6.9 M) and benzyl adenine (2.2, 4.4, 6.6, and 8.8 M) at different concentrations on MS medium. As compared to either IAA or BA alone, the frequency with which leaf explants respond to the stimulus to regenerate is considerably boosted when the two are used together. The addition of BA (4.4 M) and IAA (1.71 M) to the growing medium results in the maximum number of shoots (121.15) and the greatest shoot length (3.10.73) within 3-4 weeks of cultivation. Transfer the shoots to MS media that has been treated with IBA (2.46 M) [78] once they have had time to develop. Successful shoot induction from juvenile Scoparia dulcis leaf explants requires the addition of two cytokinins (2.32 M KI and 4.45 M BAP), 2.85 M IAA, 10% CM, and 1483.79 M adenine sulphate to MS medium. A single explant of a juvenile leaf produced 59 new shoots in just 13 days. When KI and BAP were combined in the growth media, blooming was triggered [53]. 6-Benzyl amino purine (BAP) was added to MS medium at concentrations of 13.2, 17.6, and 22.2 M, and then explants were treated with either 0.5 M or 2.8 M indole-3-acetic acid (IAA) or 2.6 M naphthalene-3-acetic acid (NAA) for various amounts of time. Overall, the greatest number of shoots (14.01.14) and the longest shoot length (2.970.18) were produced by media containing both BAP (22.2 M) and IAA (0.5 M). Callus formation mediated regeneration, and when BAP was used in conjunction with NAA, fewer shoots were produced per explant than when IAA was used. Separated long-stalked shoots were transplanted into MS media containing indole-3-butyic acid (IBA 4.9 M), which stimulated the development of roots [2]. The MS medium utilised to treat the explants included both TDZ and IAA. Rate of shoot multiplication was the greatest in MS medium containing 4.0 M TDZ and 1.0 M IAA. After 21 days of growth in 9 mM KIN-supplemented media, the overall length of the mature, fully formed shoots was 5.8 centimetres. The newly regenerated shoots were rooted in a half strength MS basal media containing varying concentrations of IBA and IAA. Media with 2.8 M IBA resulted in the greatest number of roots [74]. Calli grow green and compact when IAA (0.15 to 2.0mg/l) and BAP (0.10 to 2.5mg/l) are introduced to MS medium containing auxins, phytohormones, and rhustox (30 CH). While 0.50mg/l of BAP and 0.30mg/l of IAA both caused brown friable calli, neither was particularly effective. A similar dosage range (1.5-6.5mg/l) of 2, 4-D and Kinetin was equally effective in eliciting brown friable calli. Rhustox (30 CH) (100/100ml) promotes cell division when combined with 2, 4-D and kinetin (1.5/1.5mg/l) [80]. The induction of flower buds in Scoparia dulcis can be accomplished by treating leaf explants with MS media containing 13.93 M kinetin and 1.14 M IAA. 40-45 days after replanting, flowers appeared, and 50-55 days later, fruit formed [1].

2.2. Shoot Tip as Explants

A variety of solutions containing kinetin (0.1-1.0mg/l), BAP (0.1-1.0mg/l), NAA (0.1-0.5mg/l), and AgNO3 (0.1-1.0mg/l) were applied to the developing tips of Sphaeranthus indicus. The MS medium supplemented with kinetin (1.0mg/l), NAA (0.1mg/l), and AgNO3 (0.4mg/l) yielded the greatest number of shoots per plant (34.30.36). More robust, longer-lived shoots emerge after AgNO3 is added to the medium at any concentration. In comparison to the control group, the number of shoots produced by each plant was enhanced by 35% when AgNO3 was applied at a concentration of 0.4mg/l (10.8 0.12). After ethylene inhibitor, silver nitrate, and growth regulators were applied, there was a threefold rise in the number of multiple shoots and a twofold increase in the length of the shoots, compared to the control. The in vitro developed shoots were planted in rooting media with varying concentrations of auxins such NAA and IAA and AgNO3 (0.1-0.6mg/l). The combination of NAA (2.0mg/l) and AgNO3 (0.4mg/l) resulted in a higher rooting response (21.6%). Out of the three cytokinins tested (BAP, Kinetin, and TDZ), BAP was found to be the most efficient at spawning and spreading adventitious shoots. Explants with the highest frequency of responsiveness (100%) and the highest number of shoots (23.0) per explant were cultured in MS medium with 8.8 M BAP for 60 days. As soon as the cuttings were transplanted into the growth regulator-free MS media, they all began sending out new roots from the plant's base. Chlorocholine chloride (CCC) at a concentration of 6.3 M was found to be optimal for priming the microshoots of Eclipta alba [59]. To standardise micropropagation of the medicinal herb Eclipta alba (L.) Hassk, shoot tips and nodal segments were obtained from in vitro produced plants and cultivated. The explants' quick multiplication was aided by the addition of 1mg l1 of BAP to the MS medium. In a solution containing 0.5mg l-1 BAP and 0.5mgl-1 gibberilic acid, the developed shoot buds were cultivated and grew. The best outcomes were seen in MS medium supplemented with 1mgl 1IBA [13]. It was shown that MS medium combined with BAP (0.5 M) and NAA resulted in the maximum frequency (95%) and number (32,2 0.4) of shoot regenerations (0.5 M). The fresh shoots rooted most successfully in MS medium supplemented with 0.2 M IBA [36]. Different colchicine

concentrations (0%, 0.01%, 0.05%, 0.15%, 0.25%, and 0.3%; w/v) and treatment times (12, 24, 36, and 48h) were tested for their effects on the shoot tip (ST) and nodal segment. For shoot regeneration, the explants were placed in Murashige and Skoog (MS) medium with 1.5mg L 1 N6-benzylaminopurine and 0.5mg L 1 NAA; for root induction, the explants were transferred to 12 MS medium with 1.0mg L 1 indole-3-acetic acid [63].

2.3. Nodal Segments as Explants

Multiple carbon sources (1-6%), an ethylene inhibitor (0.4mg/l silver nitrate), Kinetin (1.0mg/l), and NAA (0.1mg/l) were tested for their ability to promote the formation of many shoots from axillary bud or nodal explants of Sphaeranthus indicus. The types of carbohydrates and AgNO3 used had a significant impact on the regeneration frequency, growth rate, and reproduction rate. The number of shoots grown in MS-medium containing 3% fructose and 0.4mg/l silver-nitrate was more than that of silver nitrate-free media (29.1). It was impossible for regeneration to take place because there were no carbon sources available. Sphaeranthus indicus nodal explants successfully developed several shoots when cultured on a medium containing 3% fructose, 2% sucrose, 1% maltose, and 1% glucose. Isolated in vitro shoots were then transplanted into a rooting medium containing NAA, IBA (1.0-2.0mg/l), and AgNO3 (0.1-0.6mg/l) [20]. When Sphaeranthus indicus (compositae) explants were grown in MS medium enriched with 2-4 D (2.0mg/l), the explants regenerated often and formed calluses. Shoot initiation was observed in calli subcultures cultured in MS medium supplemented with 6 Benzyl Amino Purine (BAP) at a dose of 1.0mg/l (0.3-1.5). BAP and 0.5mg/l Kn increased the number of shoots in 30-40 days. Shoots can be rooted in MS medium by adding 1.0 m/l of Indole Butyric acid (IBA) [46]. It was critical to employ appropriate medium, carbon sources, plant growth regulators, and even coconut water when cultivating Eclipta alba in the lab. To maximise shoot number and length in an in vitro plantlet production system using Murashige Skoog (MS) medium, we found that the following synergistic combination of benzyladenine (4.4 M), kinetin (4.6 M), 2-isopentenyladenine (4.9 M), gibberellic acid (1.4 M), 5% coconut water, and 3% sucrose was optimal: Indefinite quantities of uniformly healthy shoots could be created by sub-culturing cotyledonary node segments in the same media. At full strength, the rooting efficiency was 94.3%. Maximum of 9.8 M IBA in MS medium [4]. The strongest response was seen at 4.44 M when nodal explants were cultured with different doses of 6-benzyladenine (BA) in MS medium (range, 0.44-22.2 M). Furthermore, a concentration of BA as low as 0.44 M was sufficient for shoot multiplication and synchronous rooting [18]. Microshoot development was observed in nodal segments of Eclipta alba and Eupatorium adenophorum when they were cultivated in either modified MS medium or half strength MS media. Similarly, parallel roots could be induced in the same medium [6]. BAP, in combination with Kin or NAA, influenced shoot multiplication from tTCL nodal explants. MS medium containing 13.2 M BAP and 4.6 M Kin was the optimal medium for tTCL nodal explant shoot multiplication. On this medium, tTCL nodal explants responded perfectly, producing an average of 32.6 shoot buds. Rooting and shoot production from tTCL nodal explants were achieved in MS medium devoid of growth regulators [70]. After 60 days of growth in Murashige Skoog (MS) medium with 4.4 M benzyladenine, maximum shoot output was reached [15]. By growing on MS media containing 2.4 M 2-isopentyladenine, many roots were formed. Our research showed that MS medium supplemented with BA (10 M) was the most effective at breaking bud dormancy. After 30 days, each explant averaged about 23 0.57 shoots. The proliferation rate of node segments improved when they were cultured on new media containing 2 M BA, a concentration at which BA was not as toxic. After three rounds of subculturing, the maximum number of average shoots generated was 791.90. The reproduction rate did not decrease after this number of rounds. When microshoots were cultivated in MS medium diluted to half strength and supplemented with 0.5 M IBA, they rooted the most successfully [35]. Explants were grown in MS media supplemented with different concentrations of cytokinins, notably BAP and Kn, either alone or in combination with auxins (IAA/NAA), resulting in the production of several shoots. Following collection of many shoots, these were put to a medium containing varying concentrations of auxins (IAA/NAA/IBA), which prompted rooted [67]. E. alba nodal explants cultured in cytokinin-rich B5 medium regenerated and multiplied. Additional Cytokinins [6-benzylaminopurine (BAP), kinetin (KIN), thidiazuron (TDZ), gibberellic acid (GA3), and spermidine] resulted in better outcomes. The optimum growth frequency response (7.40.9 cm shoot length & 100% regeneration) was achieved at concentrations of 1.0 BAP+ 0.3 KIN+ 1.5 GA3 (mg/L). Half-strength B5 medium supplemented with IBA (1.0mg/L) hormone resulted in a 92% increase in root length (7.00.8 cm) and root density (8.80.8) [10]. Rapid axillary bud formation from a single nodal segment (NS) used as an explant source for in vitro culture establishment was achieved by using Murashige Skoog (MS) media supplemented with 1.0mgl 1 N6-benzyladenine (BA) and 0.25mgl 1 á-napthalene acetic acid (NAA). In just 21 days, using the same medium, a very high rate of shoot multiplication was achieved (22 shoots per axillary bud). MS media with 1.5mg l 1 indole-3-butyric acid resulted in the highest root development per shoot (IBA) [62]. Explants grown in MS media supplemented with either BAP and Kn alone or NAA and IAA resulted in the production of several shoots. The highest number of multiple shoots (18.40+-0.67) was

produced when MS media was supplemented with 1.0mg/l BAP and 0.1mg/NAA. Shoots were cultured in vitro on MS medium with and without IBA, IAA, and NAA. Half-strength MS with 0.1mg/l IBA was found to be the best root induction medium [79], with 96% of shoots taking root in it. Plant nodal explants from in vitro-grown Eclipta alba L. plants developed yellowish white, friable calluses after 3 weeks of growth on Murashige Skoog (MS) media supplemented with 10.75 mM -napthaleneacetic acid (NAA) and 9.04 mM 2, 4- dichlorophenoxyacetic acid (DOCA) (2,4-D). Only after an additional four weeks of growth in MS, basal media were half of the calluses able to transform into somatic embryos. Somatic embryo development did not occur when calluses developed on nodal explants cultured in MS medium enhanced with indole-3-acetic acid (IAA) were transferred back into MS baseline media. Somatic embryos were used to create plantlets, which were then grown to maturity. These results demonstrate the high competence for somatic embryogenesis of Eclipta alba nodal explants. After three weeks in culture on Murashige Skoog (MS) media supplemented with 10.75 M -napthaleneacetic Acid (NAA) and 9.04 M 2, 4-dichlorophenoxyacetic acid (DCPA), nodal explants from in vitro-grown Eclipta alba L. plants formed yellowish white, friable calluses (2, 4-D). About half of the calluses were successful in developing into somatic embryos after being incubated in MS basal media for four weeks. Though a callus did emerge from nodal explants cultured in MS medium supplemented with indole-3-acetic acid (IAA), replanting the callus into MS basal media did not result in the formation of somatic embryos. Somatic embryos could be transformed into plantlets and then raised to maturity. These findings suggest that nodal explants from the Eclipta alba plant can successfully go through the process of somatic embryogenesis [11].

2.4. Leaf and Nodal Segment as Explants

Leaf and nodal segments of field-grown plants were aseptically cultured on agar solidified MS medium supplemented with different concentrations and combinations of three PGRs, namely IAA, NAA, and BAP, to develop an efficient micropropagation protocol in Scoparia dulcis Linn., a crucial medicinal plant. When cultivated in MS media supplemented with 0.5-2.0mg/l BAP alone or in combination with 0.5-1.0mg/l IAA or NAA, both explants formed a compact, green callus. On MS, a combination of 1.5mg/l BAP and 0.5mg/l IAA resulted in the highest levels of callus development for all explant types. When cultivated on media supplemented with various PGRs, these callus tissues differentiated and produced several shoot buds (BAP, IAA, and NAA). The medium with 1.5m/l BAP+ 0.5mg/l IAA produced the most shoot buds (28.1%0.30%). Numerous shoot buds were shown to rapidly elongate on elongation media, with the maximum elongation (6.2 0.10) occurring on MS containing 1.5mg/l BAP+ 1.0 mg/l IAA. The extended shoot buds rooted properly in the rooting medium. MS medium lowered to half strength and supplemented with 1.0mg/l IBA+ 0.5mg/l IAA facilitated more root induction and proliferation compared to undiluted MS media alone [41].

2.5. Shoot Tips and Nodal Segments as Explants

Multiple shoots could be induced by growing shoot tips and nodal segments on MS medium with cytokinins added at various concentrations. After 8 weeks in culture, the nodal segment produced the most shoots (85.3 per explant) on MS media supplemented with 1.0mg/l BAP. With 0.5mg/l of NAA in the MS media, the root-to-shoot ratio increased from 57 to 21.

2.6. Leaves and Nodes as Explants

Scoparia dulcis was successfully micropropagated at scale in 2 4-dichloro phenoxyacetic acid and 6-benzylamino purine (1.5mg/L each) enriched Murashige skoog (MS) media. The highest number of shoots was achieved when BAP and IAA (at 1.5mg/L each) were combined. Using five distinct subcultures of isolated shoots, we were able to achieve multiple shot proliferation. Four weeks after being transplanted onto MS solid and liquid medium, which favoured rooting, the majority of the transplanted shoots (4-5 cm) had rooted and established (70-85%) [61].

2.7. Nodes as Explants

For direct plant regeneration, single node explants were inoculated on MS media supplemented with 3% (w/v) sucrose and varied concentrations and ratios of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and Naphthalene acetic acid (NAA) (NAA). In a four-week culture, most shoots emerged when the medium contained 0.5mg/l BAP and 0.25mg/l IAA (22). For direct plant regeneration, single node explants were plated in MS media supplemented with 3% (w/v) sucrose and varied concentrations and ratios of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and Naphthalene acetic acid (NAA) (NAA). The medium containing 0.5mg/l BAP and 0.25mg/l IAA resulted in the greatest number of shoots after four weeks in culture (22). The rooted cuttings were cultured on MS medium with 0.5mg/l of IBA for three weeks after they were separated. In vitro flowering and shoot regeneration from nodal explants were observed on MS media with 0.5mg/l KN and 2.0mg/l IAA [34]. BAP is the superior phytohormone for shoot regeneration compared to the combination with auxin, as shown by in vitro mass propagation of this plant using nodal explants yielding an

optimal result of 8.25 in MS media supplemented with 1mg/l BAP. When using MS medium supplemented with 0.5mg/l IBA, the optimum root induction concentration was found to be 12.50, while when using MS medium supplemented with 0.75mg/l IBA, the concentration was found to be 7.25. This means that when it comes to regenerating shoots, IBA alone is the most efficient phytohormone [33].

2.8. Apical and Axillary Buds as Explants

Explants grown in MS basal medium supplemented with 0.5mgl-1 BAP+0.1mgl-1 NAA resulted in the production of 18 shoots in 94% of cultures. Each culture spawned 26 offshoots after being subcultured numerous times in the same medium, and they quickly spread. Rooted shoots were grown in a petri dish with a medium containing 1.0mgl-1 IBA and 1.0mgl-1 NAA [24]. Newly emerging plants provided the explants from their apical and axillary buds. The best medium for stimulating shoot growth was determined to be MS basal media supplemented with 0.1mg/l BAP, where 94% of explants produced 12 shoots per culture. Repeatedly subculturing in the same media led to rapid shoot multiplication, with 16 new shoots each culture. Within 4 weeks of culture on half strength MS media with 0.5mg/l IBA + 0.5mg/l NAA [25], the highest root initiation frequency (85.20%) and number (13.40) were observed.

2.9. Seeds as Explants

Researchers looked at what would happen if they added BAP (2mg/L) and NAA (1mg/L) to Murashige Skoog (MS) media for in vitro plantlet development. The rapid growth of the plants could be attributed to the growth medium. We were able to keep a healthy supply of fresh shoots growing by re-culturing pieces of the mature plant on the same medium. Each plantlet grew into a full, healthy, rooted plantling [66].

3. Phytochemical Aspects of Some Wet Land Plants

For this purpose, we use the term "phytochemical" to refer to any chemical that is a result of a plant's primary or secondary metabolism. Nitrogen compounds, fatty acids, terpenoids, flavonoids, alkaloids, and other carbohydrates are all included. Sphaeranthus indicus contains many beneficial phytochemicals, including essential oil, sesquiterpene lactones, eudesmenolides, flavanoids, and phenolic acids [16]. The powder was extracted from the dried plant components using solvents such petroleum ether, benzene, chloroform, ethanol, and triple-distilled water. Carbohydrates, amino acids, proteins, fixed oil, flavonoids, terpenoids, and alkaloids were discovered to be present in several extracts during preliminary phytochemical analysis [3]. The primary secondary metabolites in this species were determined by phytochemical screening [21]. These metabolites included alkaloids, flavonoids, phenols, steroids, and tannins. In addition to eudesmanoids and sesquiterpenes, Sphaeranthus indicus also contains a wide variety of other phytochemicals such stigmasterol, sitosterol, geraniol, and methyl chavicol [23]. The n-hexane fraction was analysed for its phytochemical make-up and found to contain phytosterols, oils, and resins. The n-hexane fraction was analysed by gas chromatography mass spectrometry (GC-MS) [56], which identified eleven chemicals. Myristic acid, pentadecanoic acid, palmitic acid, margaric acid, stearic acid, oleic acid, elaidic acid, linoleic acid, linolenic acid, and behanic acid methyl esters are all examples. Initial phytochemical screening of Sphaeranthus indicus [17] identified and measured a variety of bioactive components, including alkaloids, flavanoids, proteins, and total polyphenols. This plant has been harvested for its several chemical compounds, including coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes, and their glycosides. This plant's extracts and metabolites have been shown to have pharmacological effects [45]. Many different types of chemicals, including coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes, and their glycosides, have been isolated from E. alba [40]. Eclipta alba contains a wide variety of compounds, including coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes, and their glycosides. Extracts and metabolites from this plant have been linked to a variety of pharmacological actions [8]. Wedelolactone, eclalbasaponins, ursolic acid, oleanolic acid, luteolin, and apigenin are just some of the phytoconstituents found in Eclipta alba plant extracts; these compounds have been studied for their individual anticancer, hepatoprotective, snake-venom neutralising, anti-inflammatory, and antimicrobial effects. Wedelolactone, a coumestan molecule, luteolin, a flavonoid, and -amyrin, a terpenoid are all present in E. alba, suggesting the plant has promising antiepileptic potential [65]. Scoparia dulcis was studied for its phytochemical potential, and a steroidal glycoside called sitosterol-D-glucoside and a flavonoid called 4, 5, 7-trihydroxy-6-methoxyflavone, or hispidulin, were isolated from an ethanol extract of the whole plant. These compounds can be used to develop new drugs to treat cancer, arthritis, gastrointestinal disorders, and skin diseases. Both compounds were originally extracted from S. dulcis [51]. Extractions of Bersama abyssinica were made using ethyl acetate, methanol, and water from the plant's original soil. Extracts of Bersama abyssinica Fresen and Scoparia dulcis L. were tested for their antioxidant properties, inhibition of enzyme activity (-amylase, -glucosidase, acetyl- and butyrylcholinesterase lipase, and tyrosinase), and phytochemical profiles. Both Scoparia

dulcis and Bersama abyssinica methanol extracts had 75.21mg rutin equivalent/g extract [68], while the aqueous extracts contained 180.62mg gallic acid equivalent/g extract [of each species]. GC-MS analysis identified six different phytochemicals, with (Z)-7-Hexadecenyl acetate (ME) and -Cyclocitral (AE) having the highest peak percentages (respectively) at 51.51% and 43.90%, respectively. Scoparia dulcis has phenolic chemicals, alkaloids, glycosides, saponins, steroids, and tannins [38]. Carbohydrates, glycosides, phenols, flavonoids, saponin, proteins, and amino acids were all found by phytochemical testing, indicating the presence of numerous bioactive components. There is, however, a trace quantity of alkaloids [44] present. The phytochemical study involved four different extracts. The phytochemical study revealed the presence of many useful compounds, including flavonoids, terpenoids, phenols, and saponin [12]. Scoparia dulcis was analysed chemically, and researchers recovered the glycosides benzoxazinone (1), phenylethanoid (2), flavone (3), and lignin (4) from the methanol fraction of the plant [5,75]. The phytochemical characteristics of ethanol extracts of Scoparia dulcis (Scophulareacea) roots and leaves were analysed. Based on preliminary phytochemical analysis, we know that the extract contains tannins, saponins, alkaloids, flavonoids, terpenoids, and phenols. No heart-healthy chemicals were found in this study, including cardiac glycosides, anthraquinones, or reducing sugars [47].

3.1. Alkaloids

Experts have provided a detailed description of the plant's biochemical components. Sphaeranthus indicus has been linked to the alkaloid spaeranthine [5].

3.2. Terpenoids and Steroids

Researchers have observed a significant percentage of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes in the essential oils of Sphaeranthus indicus [55]. The hydro-distilled Sphaeranthus indicus essential oil was analysed using gas chromatography and gas chromatography mass spectrometry. Eighty-four percent of the oil was made up of 38 distinct compounds. The top four most common compounds were 2, 5-dimethoxy-p-cymene (18.2%), -agarofuran (11.8%), 10-epi—eudesmol (7.1%), and selin-1-en-4-ol (7.1%). (12.7%) [37]. The herb includes a wide variety of active pharmaceutical compounds, including eudesmenolide-7-hydroxy eudesm-4-en-6, 12-olide, 2-hydroxycostic acid, -eudesmol, ilicic acid, and methychavicol. Pure -ionone, d-cadinene, terpinene, citral, geraniol, geranyl acetate, sphaerene, indicusene, and sphaeranthol essential oils [72]. A bicyclic sesquiterpene lactone was isolated from the petroleum ether extraction of aerial portions of the Indian Composite Sphaeranthus indicus [71]. Three essential oils of S. indicus were analysed, and a total of more than ninety-five volatiles were found; the most abundant of these were -eudesmol (21.4%), 2,5 -dimethoxy-p-cymene (16.2%), -caryophyllene (7.8%), and -cadinol (7.8%), along with floral oil (7.2%). Herb oil: 2, 5 - dimethoxy-pcymene (27.0%), T-cadinol (12.5%); root oil: 2, 5-dimethoxy-pcymene (28.3%), -cadinol (25.3%), (2) -arteannuic alcohol (10.1%), -maaliene (3.9%), and caryophyllene oxide (3.1%) [32]. Sphaeranthus indicus L. acetone extracts yielded several useful compounds, including the novel sesquiterpene lactone 7-hydroxyeudesm-4-en-6, 12-olide (1), the novel sesquiterpene acid 2-hydroxycostic acid (2), and the well-known substances -eudesmol (3) and ilicic acid (4) [73]. Two biological markers, beta-sitosterol and 7-hydroxyfrullanolide, were isolated and characterised using high-performance thin layer chromatography, melting point, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass analysis [48]. Sphaeranthus indicus has yielded several compounds of interest, including two newly discovered eudesmanolides, a third eudesmanolide, and the sesquiterpenoids cryptomeridiol and 4-epicryptomeridiol [60]. A total of fourteen compounds have been isolated from the aerial portions of Sphaeranthus indicus [64], including two novel eudesmanolide type sesquiterpenes, indicusalactone (1) and ()-oxyfrullanolide (2). By comparing their spectra to those of other 7-hydroxyeudesmanolides, the structures of three novel eudesmanoids isolated from Sphaeranthus indicus were determined [54]. They assigned novel eudesmanoids the names 11-alpha, 13-dihydro-3alpha, 7-alpha-dihydro-4, 5-epoxy-6 beta, 7-eudesmanolide (1), and 11-alpha, 13-dihydro-7-eudesmanolide. Phytochemical study of S. dulcis resulted in the isolation of two new acyclic diterpenes. Acetic acid 6-hydroxyl -2 - (6- hydroxyl -4 -methyl- hex-4- enylidene)-4, 8 -dimethyl -undeca -4, 8-dienyl ester (1) and Acetic acid 8-hydroxy -2 - (6-hydroxyl -4 -methyl- hex-4- enylidene)-4, 8 -dimethyl -undeca -4, 8-dieny (6- hydroxyl -4-methyl-hex-4-enylidene) Scopadulciol, a new tetracyclic diterpenoid, was isolated from a 70% EtOH extract of Scoparia dulcs together with 6-methoxybenzoxazolinone, glutinol, and acacetin [3]. Based on spectral evidence, it was determined to be ß-benzoyl-12-methyl-13-oxo-9 (12), 9 (12) t, -dihomo-18-podocarpanol [27]. Two new labdane-type diterpenoids, scoparicols C (1) and D (2), as well as a new scopadulane-type diterpennoid, 1-hydroxydulcinodal-13-one (3), and six biogenetically related analogues, have been isolated from the aerial parts of Scoparia dulcis (49). The molecular structures of these compounds were characterised using spectroscopic techniques such MS NMR and ECD. Compound 3 was the first of the scopadulane-type diterpenoids to have its C-1

oxidised [39], whereas compound 7 was the first of its kind to be acquired as a natural product in this study. High-performance liquid chromatography (HPLC) analysis of diterpene acids in various Scoparia dulcis plants from Paraguay revealed the presence of both scopadic acid B type (SDB) and scopadic acid A type (SDA) (SA). Both SDB and SA were found in leaves, but mostly in the latter [26]. Three labdane-type diterpene acids, scoparic acid A [1] [6-benzoyl-12-hydroxylabda-8 (17), 13-dien-18-oic acid], scoparic acid B [2] [2-hydroxylabda-8 (17)-oic acid], and scoparic acid C [3] were isolated from the active extract using bioassay-directed fractionation. With the addition of scoparic acid C [3] and [6-benzoyl-14, 15-dinor-13-oxo-8 (17)-labden-18-oic acid], [2-hydroxylabda-8 (17)-oic acid] was created. Scoparic acid A has been identified as an effective inhibitor of ß-glucuronidase [28]. The Scoparia dulcis plant yielded two new scopadulane diterpenoids [14]. Scopadulcic acids D (1, SDD) and E are the names of these substances (2, SDE). Four scopadulane-type diterpenoids (4-7), including one with the ridiculous name scopadulcic acid C, and nine additional compounds were isolated from the Vietnamese plant Scoparia dulcis L. (Scrophulariaceae) [52]. We isolated palmitic acid (1), b-sitosterol (2), glutinol (3), and b-amyrin (4) and isomultiflorenol (5) from Scoparia dulcis L. leaf extracts (5). Their structures (Scrophulariaceae) were inferred using spectroscopic methods [19]. The effects of 12-O-tetradecanoylphorbol-13-acetate (TPA), a known tumour promoter, were attenuated by the tetracyclic diterpenoid scopadulcic acid B (SDB) isolated from the medicinal plant Scoparia dulcis L.; SDB inhibited TPA's stimulating impact on skin tumour [49].

3.3. Flavanoids

Sphaeranthus indicus [43] leaves were the source of a novel flavonoid c-glycoside called 1-(5-hydroxy-7-methoxy-C)-glycosylflavone, which has been shown to have anti-inflammatory effects. Bioassay-based extract separation and purification of anti-HCV phytochemicals included in active fractions revealed the presence of two flavonoid compounds, luteolin and apigenin, and Wedelolactone [42].

3.4. Coumestan

Wedololactone [1.6%], dimethyl wedelolactone [30], and desmethyl-wedelolactone-7glucoside [30] are coumestan derivatives found in high concentrations in Eclipta alba. Hepatoprotective efficacy was evaluated by measuring changes in hexobarbitone sleep duration, zoxazolamine paralysis time, bromosulphaline clearance, serum transaminases, and serum bilirubin. The most potent hepatoprotective activity was seen in the range of coumestan wedelolactone (Ea II 00-80mg/kg) and desmethyl wedelolactone (Ea II 00-80mg/kg) with apigenin, luteolin, 4-hydroxybenzoic acid, and protocateuic acid (Ea II 00-80mg/kg) as minor ingredients [69]. The ethyl acetate-soluble fraction of the medicine Eclipta alba (L.) Hassk Asteraceae [76] yielded the coumestans wedelolactone and demethyl-wedelolactone in addition to a flavonoid and simple phenolcarboxylic acids. Elevated antibacterial activity was observed for the ethyl acetate fraction and isolated wedelolactone. Staphylococcus epidermidis, Staphylococcus aureus, and Salmonella typhimurium were the most susceptible strains. Shigella flexneri was the bacteria with the highest resistance. These results indicate that coumestans/wedelolactone may be a useful antibacterial agent [9].

3.5. Saponins

Antibacterial activity of Eclipta alba eclalbasaponin against Gram-positive and Gram-negative bacteria was investigated [58].

3.6. Quinines

Biologically relevant chemicals including naphthoquinone and hydrazine carboxamide were detected by GC-MS analysis [7].

4. Conclusions

Plants like Sphaeranthus indicus, Eclipta alba, and Scoparia dulcis stand out as exceptionally varied therapeutic options. Since many of these plants contain valuable phytochemicals, they are subject to extensive commercial exploitation. The Indian habitats were discovered to have a high abundance of these plants. Because of human activity in the name of progress, all of the plants that are normally found in the wild could one day become extinct. Plants having so many potential medical uses deserve to be safeguarded. It's important to shine a light on cutting-edge technology and the whole range of additional benefits provided by these facilities. Because of this, the practise of plant tissue cultivation holds a tremendous deal of significance. There's a lot of hope that this will lead to effective new medicines being created. Hundreds of useful plants have had protocols developed for their clonal production. This method also has the benefit of being quick and cheap to implement. The goal of this article is to provide an overview of the biochemical components of these plants and the applicability of plant tissue culture to these species.

Appendix

Figure 1. Sphaeranthus indicus

Figure 2. Eclipta alba

Figure 3. Scoparia dulcis

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