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Review Article

Bioactive Compounds via *in vitro* Culture Approach and Pharmacological Attributes of Genus *Euphorbia*: A Comprehensive Review

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Abstract: The family *Euphorbiaceae* comprises 2000 species and is listed as third among the largest flowering family. *Euphorbia* is used in traditional treatment for various diseases, including dengue fever, dysentery, diarrhea, and anemia. This review aims to collect data from various published literature sources for quick and effective cultivation of *Euphorbia* species through tissue culture and documentation of potent secondary metabolites obtained from different cultures of *Euphorbia* and its manifold pharmacological activities from various parts extracts. The data for this review were systematically collected from different scientific databases, including Google Scholar, Science Direct, PubMed, and published literature. Different secondary metabolites have been reported from the *in votro* culture of Euphorbia containing anthocyanin, saponins, tannins, sterols, flavonoids, glycosides, diterpenes, and sesquiterpene. The essential oils from extractions of the *Euphorbia* genus embraced about 80 active phytochemical constituents. The extracts and compounds exhibited different pharmacological activities, including hepato-protective, anti-fungal, anti-bacterial, and anti-cancer. Besides the pharmaceutical and importance of the genus *Euphorbia,* this report also described the methodologies of explant cultures, *in-vitro* production of biologically active compounds, and vital phytochemicals extraction from various parts of *Euphorbia*. Therefore, there is great attention for *in-vivo* studies on *Euphorbia* to further investigate and confirm their therapeutic effects for safe and effective medical use. The *in-vitro* cultivation technique needs further development, either in bioreactors or temporary immersions and shakes flasks to obtain vigorous sprouts of *Euphorbia*.

Keywords: Biological Activities, Bioactive Compounds, Pharmacological Relevance, *Euphorbia*, Essential Phytoconstituents, Disease Treated.

1. INTRODUCTION

Family *Euphorbiaceae* contains herbs, shrubs, trees, and succulent plants; both wild and cultivated plants of this family are found all over the globe [1]. Most of the species of this family chiefly existed in tropical regions but also predominantly extended into temperate zones. Most species of the family *Euphorbiaceae* produce milky latex; reported as poisonous in several species (*Euphorbia tirucalli, Euphorbia royleana, Euphorbia lathyris, Euphorbia esula, Euphorbia conitofolia Euphorbia milii, Euphorbia hirta*, *Euphorbia neriifolia*, and *Euphorbia heliscopia*) as described previously

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[2-6]. In Pakistan, the family *Euphorbiaceae* is comprised of 24 genera and 90 species. Besides this, extracts obtained from various parts of the genus *Euphorbia* proved their efficiency in relieving constipation and other gastrointestinal disorders. Moreover, its cytotoxic property bears antineoplastic compounds in the forthcoming time [7]. The traditional uses in medicine and industry of the latex obtained from *Euphorbia* species are well-known. Different members of this family are extensively used for aesthetic and medicinal purposes and cultivation has significant economic importance [8].

Euphorbia hirta has been investigated with diverse medicinal properties; conventionally, used for curing different ailments like dengue fever, diarrhea, dysentery, ulcer, asthma, bronchitis, etc. The latex obtained from *E. hirta* is extensively used in treating jaundice, anemia, and skin disorders. Similar antipyretic potential appeared in *Euphorbia neriifolia* [9]. Moreover, Awaad *et al*. [8] investigated several pharmacological activities from different parts of *E. hirta* extracts, which include antispasmodic, antifungal, antibacterial, anticatarrhal, diuretic, etc. Folk medicine in Australia utilized the latex obtained from *Euphorbia peplus* for curing keratosis and skin cancer [10, 11]. Recently, it is widely used as an essential part of many natural and medicinal products [12]. A huge number of pharmacological attributes have also been reported from *Euphorbia tirucalli* [13].

Tissue culture could be defined as the sterilized culture of cells, tissues, and organs in a controlled condition. Plant tissue culture is also known as sterile culture, in vitro culture, or axenic culture; an essential and fundamental technique in commercial and applied studies [14, 15]. Plant tissue culture media contain vitamins, micronutrients, macronutrients, and all other essential components required for normal growth and development. The pH recommended for the proper culturing of cells and tissues ranges between 5.3 and 5.8 [16]. Auxin's badly affecting the *in-vitro* morphogenesis of *Euphorbia nivulia*. Similarly, Martin *et al.* [17], studied the effect of auxins and cytokinin on hypocotyltyle culture of *Euphorbia esula*. Plant tissue culture is becoming a pressing need of the hour to conserve endangered species through clonal propagation and production of medicinally important plants on large scale in a controlled condition in a well-defined aseptic way [18, 19]**.** Extensive research has been carried out and still improvements need in the *in-vitro* culture technique in boosting the yield of secondary metabolites. In this literature review, we have discussed the efforts made by different phytochemists, botanists, pharmacologists, biochemical engineers, and tissue culturists for the improvement and establishment of the *in-vitro* cultivation approach of *Euphorbia* species. In a parallel review documented only the biological activity and triterpenoids content of the genus *Euphorbia*; however, no review analyzed the procedures for the preparation of different explant cultures, *in-vitro* production of biologically active compounds from various cultures, and enlisting the detailed pharmacological attributes of genus *Euphorbia* [20].

2. DATA COLLECTION STRATEGY

The information regarding this review was systematically collected from different scientific databases including Google Scholar, Science Direct, PubMed, and published literature. The papers selected from the base were most suitable on keywords: biological activities, pharmacological effects, *Euphorbia*, phytochemicals, and disease treated without time limitation. The 149 publications that were chosen, spanning the years 1978 to 2022, displayed remarkable findings that reflected current scientific trends at the time of publication.

3. PHYTOCHEMISTRY AND PHARMACOLOGY OF GENUS *EUPHORBIA*

Conventionally, the genus *Euphorbia* is used in treating some ailments, owing to its astonishing disease-curing properties. Recently the mesmerizing therapeutic potential of the genus *Euphorbia* has startled researchers, several pure compounds were also isolated from extracts of different plant parts [21]. The phytochemical screening of genus *Euphorbia* showed the presence of essential phytochemicals having robust therapeutic influence, other than its conventional uses it has robust medicinal properties [22], such as anti-arthritis, anti-diarrheal, analgesic, hepato-protective and antipyretics (Table 1 in Annexure I). We provided an overview of various parts of the

species belonging to the genus *Euphorbia* (Figures 1 and 2), reflecting the importance of various parts in context to their pharmacological potential.

Whereas, the applications of whole plants or various plant parts on different animals'/cell lines have been documented (Figure 3). In addition, flavonoids, diterpenoids, triterpenoids, tannins, and polyphenols were also being isolated from some species of *Euphorbia* through phytochemical screening. As far as their biological activities are concerned; phytochemical responses vary greatly, in most cases diterpenoids showed anti-cancer and cytotoxic activity; flavonoids and triterpenoids proved effective in treating inflammation and inhibiting pathogenic activities. Many natural products have been derived from *Euphorbia*; mainly including essential oils, pure compounds, and extracts with promising biological activities. About 80 phytochemicals have been reported from essential oils of *Euphorbia* species and prominent secondary metabolites [23]. Furthermore, *E. hirta* leaves extract confirmed the presence of essential vitamins in sufficient amounts, including vitamin B2, vitamin E, and vitamin C [24].

4. *IN VITRO* TISSUE CULTURE APPROACHES IN GENUS *EUPHORBIA*

Family *Euphorbiaceae* contains a large number of species; including many endangered and endemic species. Though, in vitro cultivation is limited to some specific genera having medicinal, aesthetic, food, rubber, and dye-yielding purposes [25]. *In vitro* culture is a conducive and effective technique for the proliferation and conservation of endangered species in a shorter period, mainly for those plants which are difficult to be grown by using conventional methods of cultivation and conservation [26]. Plants regenerated through *in vitro* culture possess some advantageous features over those cultivated in fields via conventional agricultural practices. For instance, the cultured-grown mountain arnica rhizome has a characteristic smell and taste, lacking in the same plant rhizome cultivated in field conditions. Several active phytochemicals were isolated from different plant parts and propagated via cell, tissue, and hair root culture methods [27]. It is immensely important to select a parent plant with a considerable amount of biologically active phytochemicals for callus formation; likewise, selecting those cell lines with higher yield [28]. Succeeding reports on cell culture, callus culture, shoot, leaf, and root culture, somatic embryogenesis, and nodal/inter nodal culture of genus *Euphorbia* (Table 2 in Annexure II).

5. CALLUS CULTURE OF GENUS *EUPHORBIA*

A callus is a mass of tissues having differentiated cells, developed under the influence of determinate hormonal control described previously [29, 30]. Propagated callus and shoot from stem pieces of *Euphorbia esula* HR lines and shoot regeneration from hypocotyls of non-HR lines. Maximum shoot regeneration was observed by inoculating the explants in a growth medium containing Murashige and Skoog (MS) basal salts, MS + vitamins, 1.11 µM 6-benzylaminopurine, 1.97 µM indole-3-butyric acid, and 3.0 % sucrose, pH 5.6–5.8. After 30 days multiple shoots developed from the stem (Figure 4).

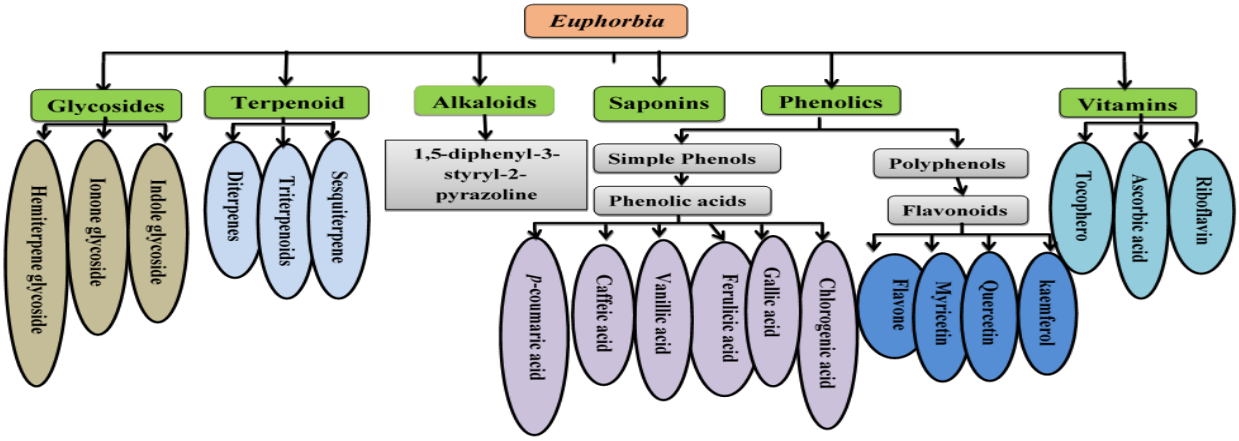


Fig. 1. Major secondary metabolites reported in *Euphorbia* species.

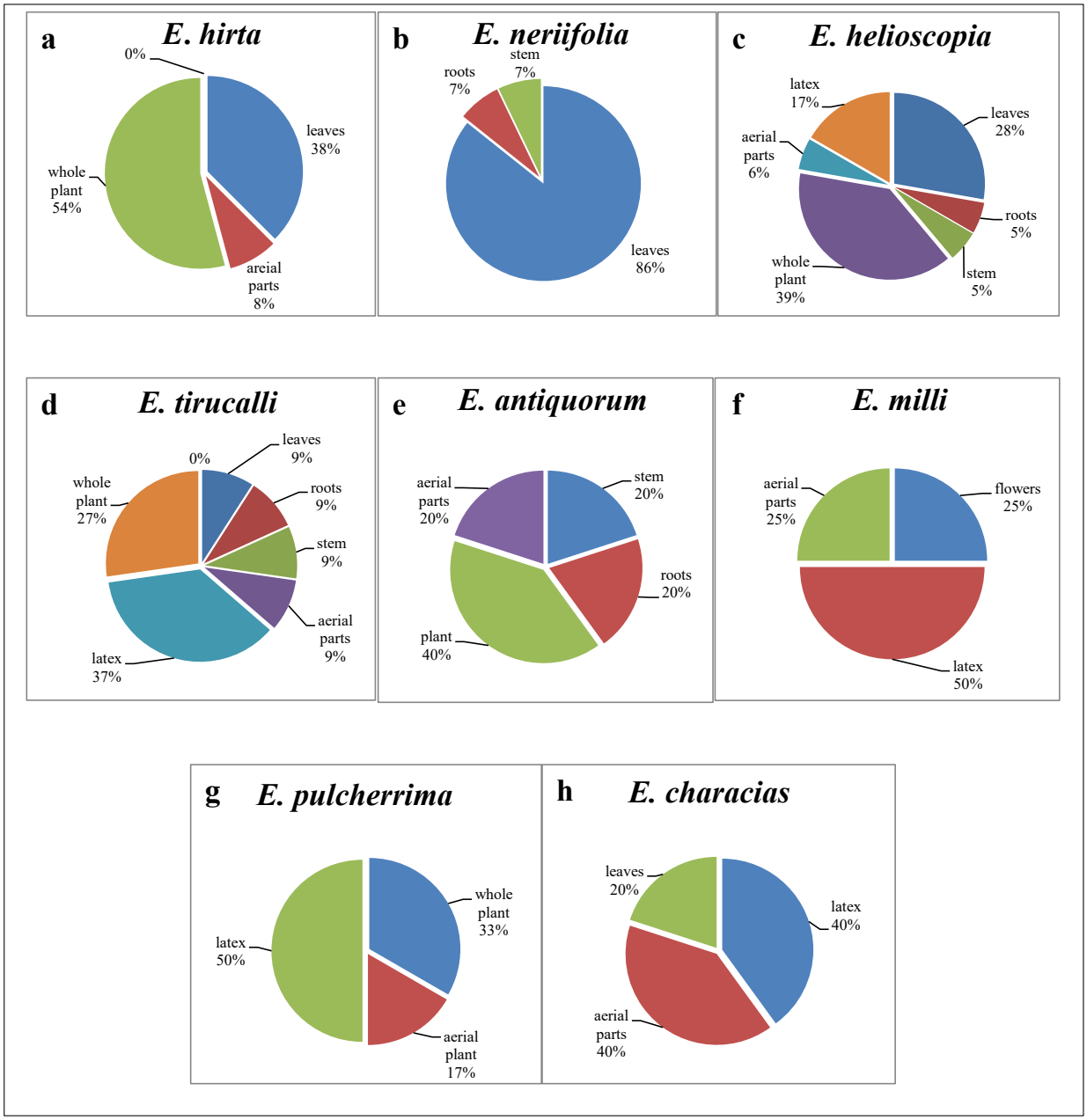
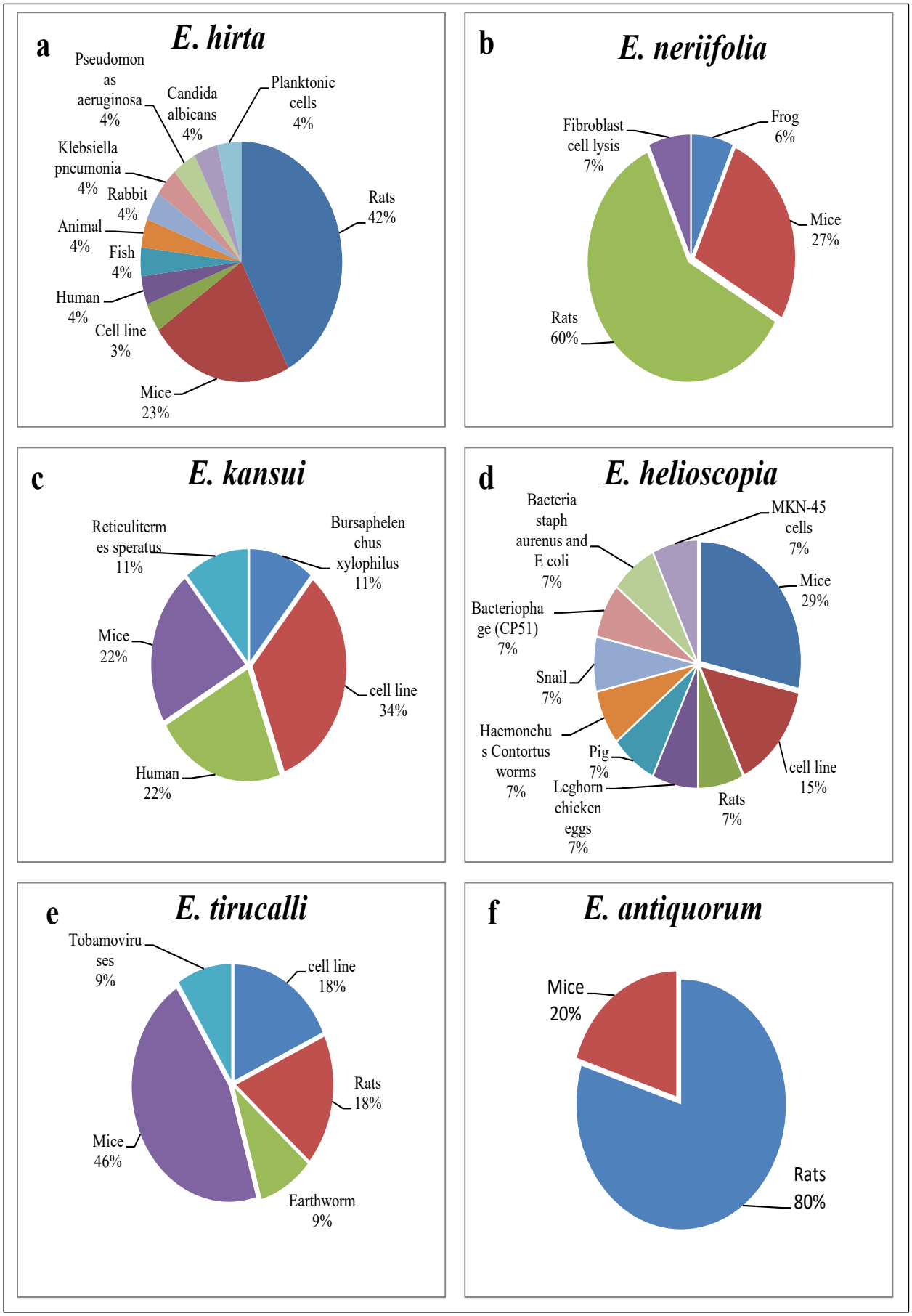


Fig. 2. Percentage of various parts of *Euphorbia* species used in pharmacology (**a**) *Euphorbia hirta* (**b**) *Euphorbia nerrifolia* (**c**) *Euphorbia helioscopia* (**d**) *Euphorbia tirucalli* (**e**) *Euphorbia antiquorum* (**f**) *Euphorbia milli* (**g**) *Euphorbia pulcherrima* (**h**) *Euphorbia characias.*

The callus culture of *Euphorbia hirta* revealed the presence of phenolic and sterol compounds with a substantial amount of chlorogenic acid (79.67 mg/100 g d. m.) syringic acid (32.57 mg/100 g d. m.) and brassicasterol (32.57 mg/100 g d. m.) by Özbilgin et al. [31] and Lone et al. [32]. Euphorbia tirucalli callus culture revealed the presence of euphol, tirucallol, and 4, 4-dimethyl sterols amount [33, 34]. The most prominent secondary metabolites extracted from cultured cells of *Euphorbia* species include cyanidin glycoside from *Euphorbia milli*. Similarly, the sitosterol, palmitic acid, and triterpenoids from cultured cells of *E. esula*, and phytosterol, tirucallol, triterpene, and euphol (Table 3 in Annexure III) extracted from callus cultured cells of *Euphorbia tirucalli* [33, 35]. *Euphorbia characias* callus culture hormonal regulation of triterpinols formation was investigated [36]. Leaf explants of *E. hirta* were cultured on MS+NAA and 6-benzylaminopurine (BAP) medium, at the onset of callus initiation, it was again subcultured on the same media with 1


a
E. hirta
Pseudomonas aeruginosa 4%
Candida albicans 4%
Planktonic cells 4%
Klebsiella pneumonia 4%
Rabbit 4%
Animal 4%
Fish 4%
Human 4%
Cell line 3%
Mice 23%
Rats 42%
b
E. neriifolia
Fibroblast cell lysis 7%
Frog 6%
Mice 27%
Rats 60%
c
E. kansui
Reticuliterm es speratus 11%
Bursaphelen chus xylophilus 11%
Mice 22%
cell line 34%
Human 22%
d
E. helioscopia
Bacteria staph aurenus and E coli 7%
MKN-45 cells 7%
Mice 29%
Bacteriopha ge (CP51) 7%
Snail 7%
Haemonchu s Contortus worms 7%
Pig 7%
Leghorn chicken eggs 7%
Rats 7%
cell line 15%
e
E. tirucalli
Tobamoviru ses 9%
cell line 18%
Rats 18%
Mice 46%
Earthworm 9%
f
E. antiquorum
Mice 20%
Rats 80%

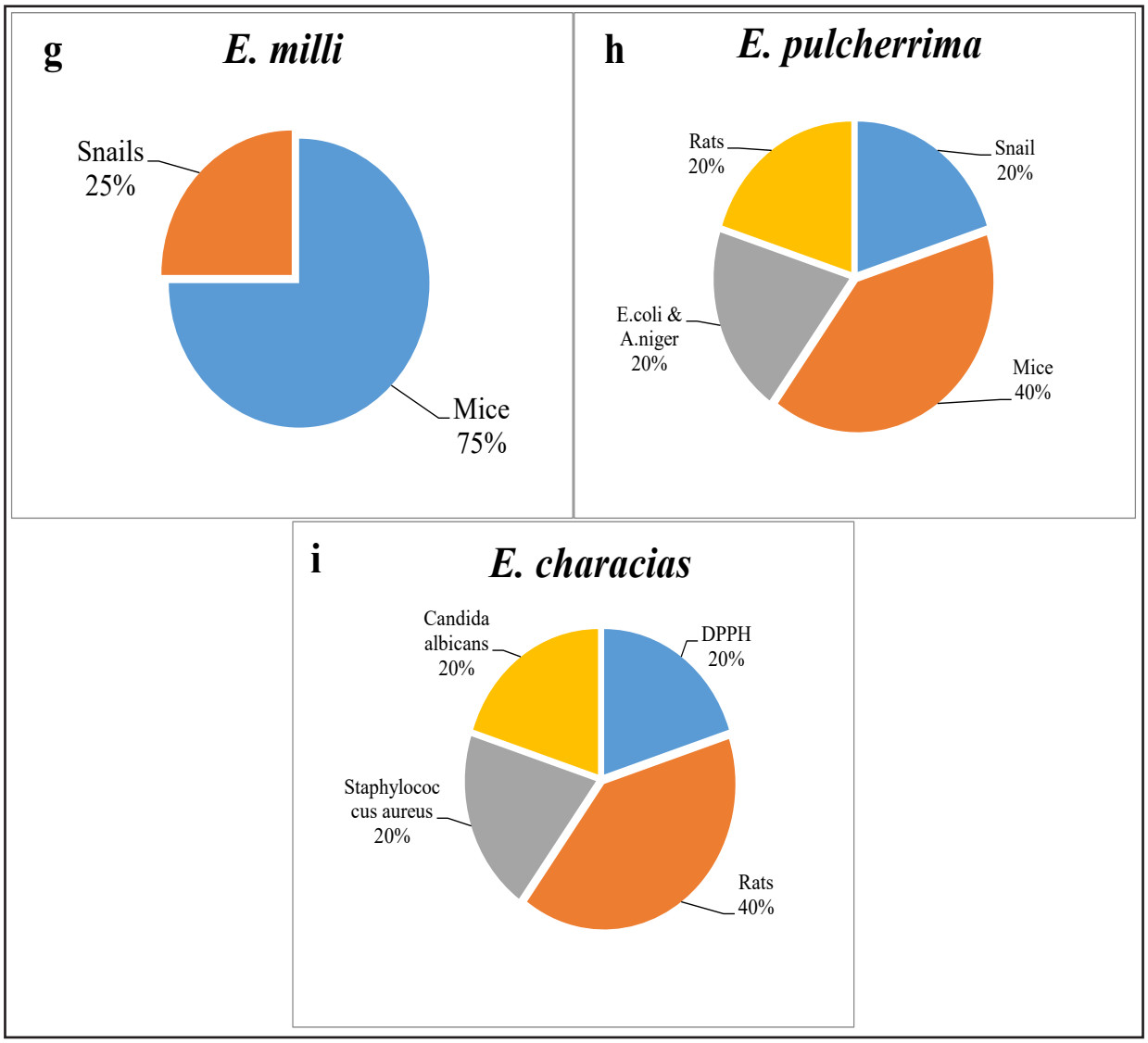


Fig. 3. Percentage of animals/cell lines used for the assessment of various pharmacological activities of euphorbia genus different parts extracts (**a**) *Euphorbia hirta* (**b**) *Euphorbia nerrifolia* (**c**) *Euphorbia kansui* (**d**) *Euphorbia helioscopia* (**e**) *Euphorbia tirucalli* (**f**) *Euphorbia antiquorum* (**g**) *Euphorbia milli* (**h**) *Euphorbia pulcherrima* (**i**) *Euphorbia characias.*

mg/L concentration of NAA (1-naphthaleneacetic acid) and BAP [30]. Red callus was produced from apical and axillary buds of *E. pulcherrima* on MS basal medium containing benzyladenine (BA) and a combination of IAA (indole acetic acid) (IAA) and BA [37]. From leaf explant of *E. helioscopia* callus was induced via Murashige and Skoog's (MS) medium supplemented with 6-benzylaminopurine [38]. Furthermore, secondary metabolite extraction is of utmost importance in culturally grown plants; making it an ideal technique for raising plants for commercial and medicinal purposes [29].

6. SHOOT, LEAF, SEED, AND ROOT REGENERATION VIA DIFFERENT CULTURES

Murashige and Skoog's (MS) + naphthaleneacetic acid (NAA) medium was used to produce roots from *E. tannensis* shoot culture, some of the seedlings were kept under glasshouse in pots containing coarse sand, peat, and perlite with a ratio of 6:3:1, respectively. The plantlets remained healthy and grew well under the glasshouse. On contrary, when placed in peat blocks all seedlings wilted and

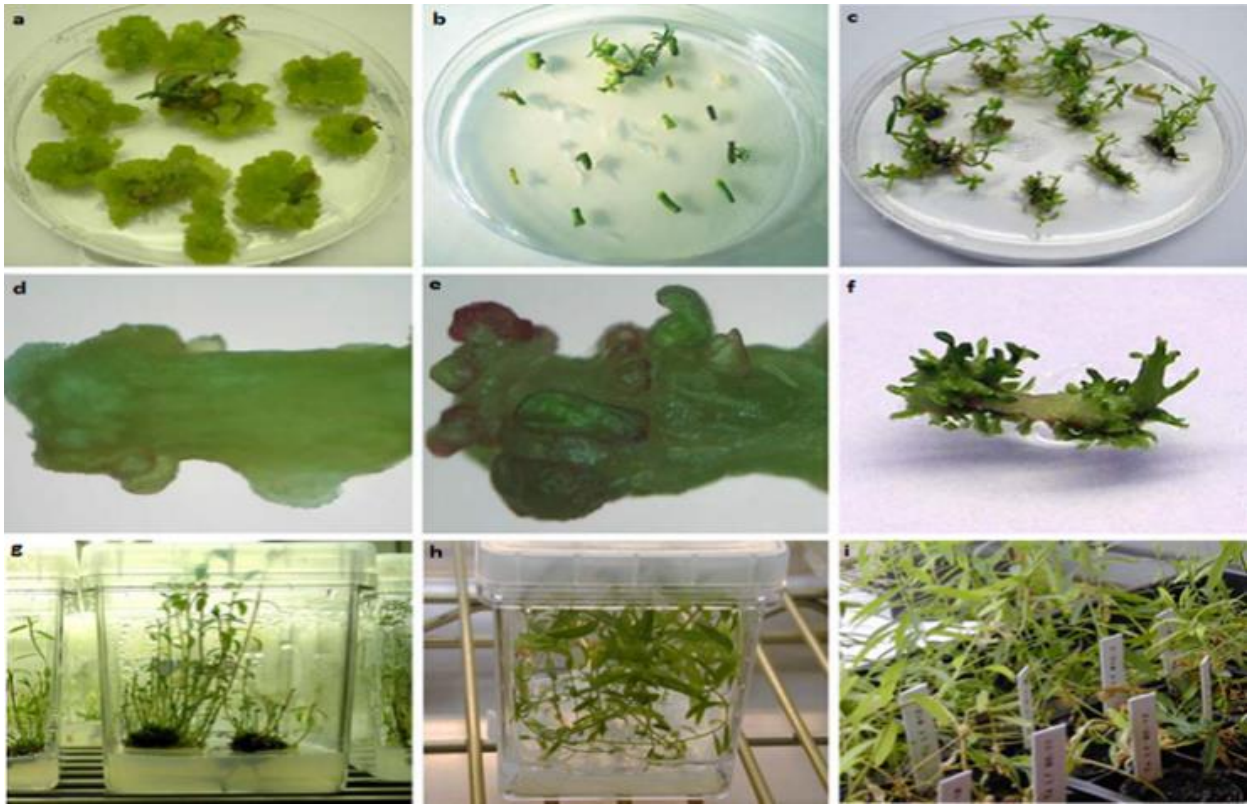


Fig. 4. In vitro regeneration of leafy spurge (*Euphorbia esula* L.) a = Large callus with a loose structure, b = Shoot regeneration from hypocotyls of non-HR lines, c = Shoots regeneration from stem pieces of HR lines, d = Callus, e = Callus and shoot primordium, f = Multiple shoots regenerated from the wounded surface of explants, g = Multiple plantlets growing from calluses, h = Plantlets developing roots in rooting medium. (i) Plantlets growing *In-vitro.*

collapsed. In comparison with *E. tannensis*; *in-vitro* root formation from shoots of *E. lathyris* was quite slow on the same medium used for the formation of roots from *E. tannensis* shoots [39]. *Euphorbia antisyphilitica* shoots were propagated via *in-vitro* culture technique by using BAP (4.44 **µ**M) and MS + NAA (0.13 **µ**M) medium, root progress was satisfactory and when transferred to the field; easily adapted to the natural climatic conditions [31]. *Euphorbia lagascae* shoots were regenerated through tissue culture technique; afterward, dipped in IBA (50 mg/L) for a period of 2 minutes, an increase of 70% to 100% survival rate was observed with the application of benzyladenine (BA) [40]. Tips of *Euphorbia pugniformis* cristate lateral shoots were cultured by using MS + NAA (0.1 mg/L) sucrose 2.0% and IBA in culture media; as a result, both of the normal and cristate types of shoots were produced. 90%-100% of cultured plantlets successfully acclimatized outside the laboratory in field conditions [41]. *In-vitro* propagation of *Euphorbia fulgens* micro shoot cuttings and their adjustment to the natural climatic conditions were established [42]. *Euphorbia pulcherrima* shoot buds were propagated through the tissue culture technique [43]. Shoots were raised from nodal shoot explant of *Euphorbia pulcherrima* (Figure 5) using a medium having 6-benzylaminopurine (BAP) in combination with adenine sulfate and GA3 (Gibberellic acid).

The induction of shoot was optimal by using BAP at 0.5 mg/L in combination with 20 mg/L adenine sulfate. Numerous roots and the highest frequency (77.8%) were observed by using MS media and supplements of 1.0 mg/L indole-acetic acid (IAA) by Sreenika *et al*. [44]. *E. esula* hypocotyl segment was applied as explant and roots were proliferated on culture media containing IAA and IBA [45]. Leaf-cultured cells of *Euphorbia milli* showed the presence of a red colour pigment mainly consisting of anthocyanin [46, 47]. Extract of leaves of *Euphorbia cotinofolia* in streptomycin possessed wide range of flavonoids, terpenoids, and steroids that help remove a pathogenic form of *Bacillus cereus, Bacillus subtilis, Escherichia coli,*

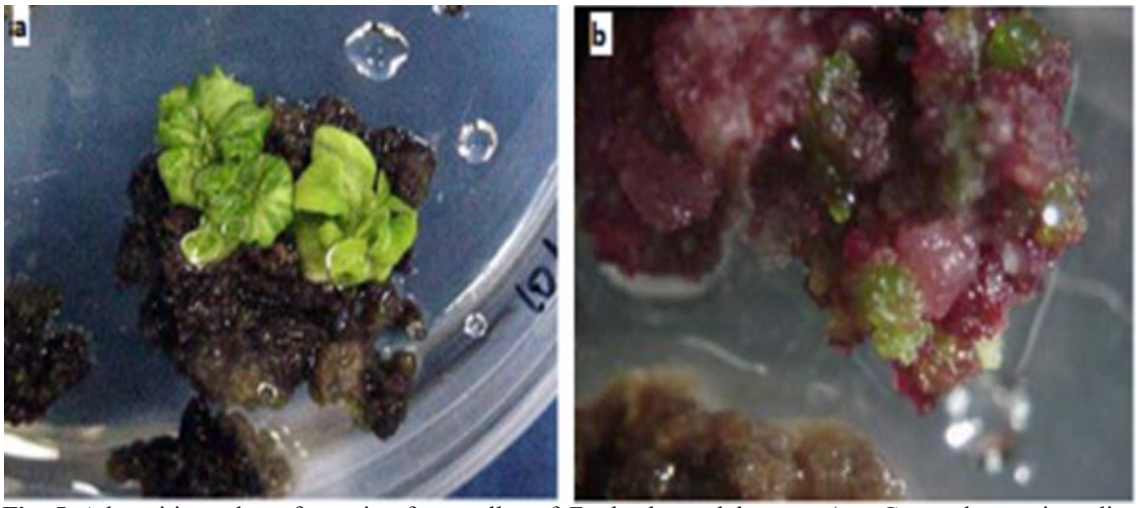


Fig. 5. Adventitious shoot formation from callus of *Euphorbia pulchurrima* (a = Green shoot primordia, b = Elongating Shoots.

Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella typhi, and *Staphylococcus aureus* [48]. Amorphous calcium phosphate nanoparticles (ACP NPs) with coumarin extracts from seeds of *Euphorbia lathyris* possessed strong cytotoxicity against colon cancer [49].

7. SOMATIC EMBRYOGENESIS NODAL AND INTER NODAL CULTURES OF GENUS *EUPHORBIA*

Cultivation through somatic embryogenesis provides a prospect to propagate those lines bearing superior quality in terms of secondary metabolites and yield attributes [43]. To date, propagation through cell suspension culture, callus culture, and somatic embryogenesis was described in several *Euphorbia* species. The somatic embryogenesis of *Euphorbia pulcherrima* was investigated [42]. From the hypocotyl of *Euphorbia pulcherrima* somatic embryo formation was reported by Biesboer *et al*. [50]. *Euphorbia tirucalli* internodal explants (Figure 6) were propagated via *in-vitro* culture technique by using Linsmaier and Skoog's (LS) + TDZ (0.02 mg/L) culture medium [51].

Euphorbia pulcherrima nodal explants were cultured on NAA + MS, isopentenyl adenine (2-iP), and Kin media [52]. *Euphorbia pulcherrima* nodal explant on MS + α-naphthalene acetic acid and isopentenyl adenine (2-iP) medium gave rise to somatic embryos [53]. Internode explant of *E. hirta* gave rise to a higher number of somatic embryos on BAP and Kin media supplemented with indole-acetic acid (IAA) and naphthalene acetic acid (NAA). NAA proved more productive than indole-acetic acid (IAA) with a higher percentage of somatic embryos. 100% response was noticed by using MS in combination with 0.5 mg/L naphthalene acetic acid (NAA), 0.4 mg/L each of BAP, and kinetin (Kin). Medium with IAA in place of NAA gave maximum (92%) somatic embryo induction [54, 55].

8. INFLORESCENCE TISSUE CULTURE

Das *et al.* [56] manipulated the inflorescence of explants (*Euphorbia milli)* for their *in-vitro* propagation. Consequently, vegetative meristems were cultivated from the meristems of an inflorescence of the main axis after one week of inoculation on MS medium supplemented with indole-3-butyric acid (IBA) and benzyl adenine (BA). Among various growth regulators, MS medium with 1.0 mg/L BA and 0.3 mg/L IBA responded better in terms of callus initiation with a maximum percentage of leaf and shoot developed.

9. CONCLUSIONS

In this review, pharmacological activities, methodologies of preparing different explant cultures, *in-vitro* production of biologically active compounds, and vital phytochemicals extracted from various parts of the genus *Euphorbia* has been documented. For successful *in-vitro* propagation of seedlings; media composition, plant growth regulators (PGRs) selection, and other vital requirements such as temperature, light, and pH are immensely important to be determined. Moreover,

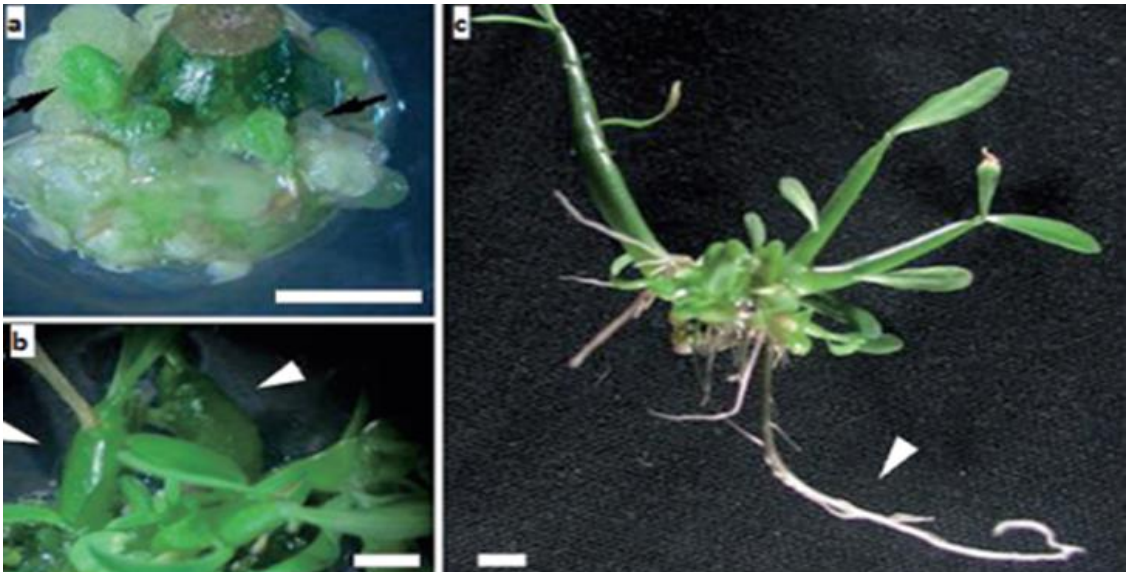


Fig. 6. Plant regeneration from internode segments of E. tirucalli. (a = Adventitious buds, b = Trunk-shaped shoots c = whole plantlet.

the plants regenerated through tissue culture need to be assessed for their safety in living organisms is also necessary for higher marketability and significance. Besides this, investigating the potential of preventing diseases by applying *Euphorbia* should be a relevant issue. Disease prevention methods and modern cultivation practices for *Euphorbia* need to be further elucidated with the application of advanced techniques like hydroponic, aeroponic, and plugged seedling culture methods that should be adopted for rapid growth and better quality of *Euphorbia* species. Still, there is a great need for an imperative propagative method and an immense desire for the swift proliferation of superior qualities of *Euphorbia* species.

10. FUTURE PERSPECTIVES AND RECOMMENDATIONS

The identification and isolation of novel bioactive chemicals from the genus *Euphorbia* should be the focus of future study. Analytical advances such as mass spectrometry and nuclear magnetic resonance can help with the isolation and structural elucidation of previously unknown substances. Further optimisation of *in vitro* culture conditions is required to maximise bioactive chemical synthesis. This includes optimising nutrient formulas, growth regulators, and culture medium components to mimic the natural environment and increase target compound yield. Exploring biotechnological technologies for large-scale synthesis of bioactive substances, such as plant cell and tissue culture, can provide a sustainable and controlled supply. This could include the development of bioreactor systems and commercial scale-up initiatives. More thorough pharmacological investigations on isolated chemicals from *Euphorbia* species are required to fully grasp their medicinal potential. Following clinical trials, their efficacy, safety, and prospective applicability in treating various diseases can be validated.

Promote interdisciplinary collaborations between geneticists, pharmacologists, biotechnologists, and other experts. Such collaborations have the potential to speed research and bring varied perspectives to bear on the issues connected with the study of *Euphorbia* species as well as establish standardized protocols for the extraction of bioactive compounds from *Euphorbia* plants. This will assure uniformity in research outputs and make it easier to compare results across investigations. Finally, the possibilities for future study on bioactive substances from the genus *Euphorbia* are encouraging, with opportunities for scientific improvements, biotechnological innovations, and sustainable practices. Implementing the recommended procedures can aid in the discovery of novel treatments, the conservation of plant species, and the promotion of responsible resource use.

11. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ANNEXURE I

Table 1. Various pharmacological attributes of the genus *Euphorbia.*

Species	Part used	Extract type	Pharmacological activities	Study type	Dose	Animal model/Cell line	References
E. hirta	Whole plant	AqE	Anti-arthritis	*In-vitro*	50 mg/kg	Rats	[57]
	Leaves	AqE	Immunomodulatory		50 g/kg	Fish	[58]
	Whole plant	HAE	Anxiolytic		200 mg/kg	Rats	[59]
	Whole plant	EtOH	Anti-nephrotoxicity		400 mg/kg	Rats	[60]
	Leaves	AqE	Anti- gastrointestinal motility		100 mg/kg to 1000 mg/kg	Rats	[61]
	Whole plant	EtOH	Wound Healing		2% w/w	Rats	[62]
	Whole plant	EtOH	Bronchodilator		200 mg/kg	Animal	[31]
	Leaves	MeOH	Acute oral toxicity		100 mg/mL to 0.07 mg/mL	Mice	[34]
	Whole Plant	EqOH	Anti-dengue		12.5 µg/mL	Human	[63]
	Whole Plant	EtOH	Anti-anaphylaxis		100 mg/kg to 1000 mg/kg	Mice	[64]
	Leaves	AqE	Anti-diabetic		300 mg/kg	Rats	[65]
	Whole Plant	AqE	Sedative		100 mg/kg	Mice	[66]
	Whole Plant	MeOH	Anti-biofilm		0.25 mg/mL	Planktonic cells	[67]
	Whole plant	EtOH	Wound healing		2% W/W cream.	Albino rats	[62]
	Aerial parts	MeOH	Anti-bacterial		0.25 mg/mL to 0.5 mg/mL	*Pseudomonas aeruginosa*	[67]
	Leaves	EtOH	Anti-bacterial		1.25 µg/mL to 200 µg/mL	*Klebsiella pneumoniae*	[68]
	Whole plant	EtOH	Anti-cancer		200 µg/mL	Cell lines	[69]
	Whole plant	Aq	Anti-inflammatory		10 µg/mL	Rabbit	[70]
	Whole plant	EtOH	Anti-allergic		0.3 mg/g	Mice	[71]
	Aerial parts	MeXOH	Antidiarrheal		50 mg/kg	Mice	[5]
	Leaves	MeOH	Anti-tumors		200 mg/kg	Rats	[72]
E. neriifolia	Leaves	HAE	Analgesic	*In-vitro*	400 mg/kg	Frog	[73]
	Stem	MeOH	Hepatoprotective	Laboratory	2.0 g/kg	Mice	[74]
	Leaves	DMSOE	Anti-cancer	*In-vitro*	500 µg/mL	Mice	[75]
	Leaves	HAE	Immunomodulatory		400 mg/kg	Rats	[76]
	Leaves	HAE	Anti-ulcer		400 mg/kg	Rats	[77]
	Leaves	EtOH	Anti-diabetic		400 mg/kg	Rats	[78]
	Leaves	EtOH	Anti-anxiety		400 mg/kg	Mice	[79]
	Leaves	HAE	Hematological		400 mg/kg	Rats	[51]
	Leaves	HAE	analgesic	Laboratory	400 mg/kg	Rats	[80]
	Latex	EF	Anti-inflammatory	-	500 mg/mL	Rats	[81]
	Roots & Leaves	EtOH	Anti-thrombotic	*In-vitro*	2.0 mg/kg	Rats	[82]
	Leaves	EtOH	Wound healing	Laboratory	200 mg/kg and 400 mg/kg	Rats	[83]
	Leaves	AqE	Anti-scorpion venom		0.706 mg/mL	Fibroblast cell lysis	[84]
	Leaves	HAE	Immunomodulatory		400 mg/kg	Rats	[85]
	Leaves	HEE	Anti-DENA-Induced Renal Carcinogenesis	*In-vivo*	50 mg/kg	Mice	[86]

Species	Part used	Extract type	Pharmacological activities	Study type	Dose	Animal model/Cell line	References
E. kansui	Roots	EtOH	Anti-nematode	*In-vitro*	5 µg	*Bursaphelenchus xylophilus*	[87]
	Roots	EtOH	Anti-proliferative		8.7 µg/mL	Cell lines	[88]
	Roots	EtOH	Hepatotoxic		8 µg/mL	Cell lines	[89]
	Roots	CdE	Anti-HIV		500 µg/mL	Human	[90]
	Roots	EtOH	Anti-cancer		50 µg/mL	Human	[91]
	Roots	DCME	Anti-cancer		2.0 µmol	Mice	[92]
	Roots	EtOH,	Anti-termites		50 *µ*g/mL	*Reticulitermes speratus*	[93]
	Roots	EtOH	Anti-obesity		100 mg/kg	Mice	[94]
	Roots	EtOH	Cytotoxic	.....	30.67 µg/mL	Cell lines	[95]
E. helioscopia	Whole plant	EAE	Anti-cancer	*In-vivo*	200 µg/mL	Mice	[88]
	Whole plant	EAE	Anti-cancer	*In-vitro*	200 µg/mL	Cell lines	[96]
	Whole plant	EAE	Anti-asthmatic		30 mg/kg	Pig	[97]
	Leaves and latex	MeOH	Anti-angiogenic	*In-vivo*	200 µg/L	Leghorn chicken eggs	[98]
	Plant	MeOH & AqE	Anthelmintic		50mg/mL	*Haemonchus vontortus*	[99]
	Leaves and stem	MeOH	Molluscicidal	Laboratory	10 ppm to 100 ppm	Snail	[100]
	Whole plant	MeOH	Anti-viral	.....	0.125 mg/mL	Bacteriophage (CP51)	[101]
	Aerial parts	MeOH	Vasodepressor	.....	2.0 mg/kg	Rats	[102]
	Leaves	MeOH	Anti-pyretic	*In-vitro*	300 mg/kg	Mice	[103]
	Leaves and latex	MeOH	Anti-bacterial		250 mg/mL	Bacteria (*S. aureus* and *E. coli*)	[104]
	Leaves and latex	MeOH	Anti-oxidant		1200 mg/kg	Mice	[96]
	Whole plant	EAE	Anti-cancer		200 mg/mL	Cell lines	[105]
	Roots	AqE	Anti-tumor		4.0 mg/mL	MKN-45 cells	[106]
	Whole plant	EtOH	Insulin secretagogue		10 µg/mL	Mice	[107]
E. tirucalli	Stem	EtOH	Anti-cancer		300.70 µg/mL	Cell lines	[108]
	Leaves	AqE	Anti-cancer		200 µg/mL	Cell lines	[109]
	Whole plant	EAE	Anti-inflammatory	*In-vivo*	10 mg/kg	Mice	[110]
	Latex	AqE	Analgesic	.....	300 mg/kg	Mice	[111]
	Whole plant	BET	Anti-arthritic	*In-vivo*	2000 mg/kg	Mice and rats	[112]
	Aerial parts	AqE	Hepatoprotective		150 mg/kg and 250 mg/kg	Rats	[113]
	Latex	PEE & DCME	Antiviral	.....	150 ppm	Tobamoviruses	[114]
	Whole plant	MeOH	Antinociceptive	*In-vivo*	30 mg/kg	Mice	[115]
E. antiquorum	Roots	AqE	Anti-diabetes	*In-vitro*	400 mg	Rats	[116]
	Whole plant	A-EtOH	Analgesic	Laboratory	500 mg/kg	Mice	[117]
	Whole plant	AEA & AqE	Anti-arthritic	.....	400 mg/kg	Rats	[118]

Species	Part used	Extract type	Pharmacological activities	Study type	Dose	Animal model/Cell line	References
E. millli	Flower	EAE	Anti-cancer	*In-vivo*	200 mg/kg and 400 mg/kg	Mice	[119]
	Aerial parts	MeOH	Sedative	.....	10 ppm and 20 mg/kg	Mice	[120]
	Aerial parts	MeOH	Antinociceptive	.....	50 mg/kg, 100 mg/kg and 150 mg/kg	Mice	[121]
	Latex	AqE	Molluscicidal	.....	22 ppm (mg/L)	Snail	[122]
E. pulcherrima	Latex	AqE	Molluscicidal	.....	0.02 mg/kg and 0.09 mg/L	Snail	[123]
	Latex	AqE	Anti-convulsive	Laboratory	250 mg/kg, 500 mg/kg and 1000 mg/kg	Mice	[124]
	Aerial parts	MeOH	Analgesic effect	.....	50 mg/kg,100 mg/kg and 150 mg/kg	Mice	[125]
	Whole plant	AqE and EtOH	Antimicrobial	*In-vitro*	2000 µg to 5000 µg	*E. coli* and *A. niger*	[126]
	Latex	AqE	Anxiolytic effect	.....	250 mg/kg, 500 mg/kg and 1000 mg/kg	Mice	[127]
	Whole plant	MeOH	Hepatoprotective	.....	1000 mg/kg	Rats	[67]
E. characias	Latex	MeOH	Antioxidant	.....	25 µL	DPPH	[128]
	Aerial parts	MeOH	Anti-inflammatory	*In-vivo*	100 mg/kg	Rats	[32]
	Leaves	EtOH	Anti-bacterial	....	1250 *µg*/mL	*Staphylococcus aureus*	[36]
	Aerial parts	MeOH	Wound healing	*In-vivo*	.....	Rats	[129]
	Latex		Antifungal	*In-vitro*	62.5 *µg* protein/mL	*Candida albicans*	[130]

ANNEXURE II

Table 2. A list of explants used growth regulators and reported basal media for *in-vitro* propagation of *Euphorbia* species.

Species	Explant	PGRs	Culture Medium	Responses	References
E. esula	Stem explant	BA + IBA + 3% sucrose + vitamins	MS	Shoot	[29]
	Hypocotyl segment	IAA + 2,4-D	B5	Shoots and Roots	[131]
	Root tissue	NAA + KIN + IAA	MS	Callus	[35]
	Cell suspension culture	2,4-D+NR	B5	Roots	[132]
	Hypocotyl segments.	IAA + polyamines	B5	Roots	[133]
	Hypocotyl segments.	Putrescine	B5	Roots and shoots	[45]
	Stem callus	Fluorescent light	MS	Cell suspension	[131]
E. lagascae	Axillary shoots	IBA or NAA	MS	Roots	[40]
E. tirucalli	Stem	BA, NAA, 2,4-D	MS	Callus	[134]
	Internode	TDZ, NAA	LS medium	AB	[135]
	Internode	TDZ	LS	AB	[54]
E. milli	Apical bud	Paclobutrazol + Sucrose + LEDs	MS	Inflorescence	[56]
	Buds	BA+ IAA+ Sucrose + vitamins	MS	Roots	[136]
E. pulcherrima	Nodal shoot segments	BAP+ GA3+AS	MS	Shoot	[44]
	Apical buds and axillary buds	IAA+BA	MS	Callus	[137]
	Shoot tips	BA + sucrose (3%) + agar (75%)	MS	Shoot	[138]
	Nodal explant	2ip + NAA	MS	SE	[139]
	Shoot	BA+ sucrose (3%) + agar (75%)	MS	Shoot	[46]
	Terminal buds and Leaf tissue	IAA + BA	MS	Callus and Shoot	[53]
	Stem nodes	NAA +2-Ip	MS	SE	[26]
	Hypocotyle segment	IAA	MS	SE	[140]
	CS	2,4-D + BA	MS	SE	[141]
	Stem nodal explants	BAP + NAA	MS	Shoot	[142]
	Petiole explants	NAA + BA+ KIN +2,4-D+IBA+IAA	MS	Buds	[142]
E. pugniformis	Tip explants	NAA + BA + sucrose	MS	Shoot	[41]
E. antisyphilitiea	Shoot	BA + NAA	MS	Axillary shoot	[143]
E. nivulia	Mesophyll cell	NAA	MS	Shoot	[17]
E. hitra	Stem explants	BAP + KIN + NAA	MS	SE	[55]
	Leaf bits	NAA+ BAP	MS	Callus	[30]
E. helioscopa	Leaf discs	2,4-D	MS	Callus	[38]
E. lathyris	Apical shoot	NAA	MS	AS	[39]
	Nodes and internodes	NAA+ BA	MS	Shoot and callus	[144]

ANNEXURE III

Table 3. *In-vitro* production of secondary metabolites from *Euphorbia* species via using different approaches.

Secondary Metabolites	Species	Culture Types	PGRs	Medium	References
Phytosterols	E. milli	LC	2,4-D, NAA, ME	MS	[145]
Triterpenol			Auxin, 2,4-D, NAA, YE		
Anthocyanin			2,4-D, NAA, CH		
		CC	2,4-D, NAA		[146]
		LC	2% sucrose, 0.8% agar, 2,4-D		[146]
		SC	MS, LS, GA, NN, HE, ME, sucrose,		[147]
Phytosterols	E. triucalli		ME, 2,4-D		[63]
Fatty Acids			YE, 2,4-D		
Anthocyanin			ME, 2,4-D		[50]
Triterpenol			ME, 2,4-D		
Triterpenol	E. characias	CC	KIN, BA, ZEA, 2,4-D		[52]
Taraxerol	E. hirta		NAA, BAP		[34]
Triterpenoid		CSC	2,4-D+BAP+NAA		[148]