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Antioxidants from Callus Technology

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Authors' contributions

This work was carried out in collaboration among all authors. Author GAI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FBJ and KJT managed the analyses of the study. Author ASK managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Antioxidants are very important compounds that are very vital in human health and they have been proven to reduce the risk of diseases such as cancer in human health. Many researchers have used callus to produce antioxidant and most of them used different techniques to get reasonable amounts of antioxidants. The technique used determines the number of antioxidants that will be produced from any explants. Callus Technology involves the techniques of producing callus and metabolites in the presence of explants using different plant hormonal combination in media, different environmental culture condition (light, relative humidity and tempreture), use of elicitors and under a sterile conditions. Callus technology is very promising due to its ability to produce a larger quantity of metabolites (antioxidants) compare to the raw extract of its explants. The use of callus to produce antioxidants is very important and very useful in discovering new plants as a source of antioxidants. The use of callus technology was reviewed for production of antioxidant from the callus of the following plants: *Sericostoma pauciflorum*, *Helicteres angustifolia L*, *Lepidium sativum L*, *Randia echinocarpa*, *Andrographis paniculata Nees*, *Citrullus colocynthis*, *Rauwolfia vomitoria Afzel*, *Decalepis hamiltonii*, *Bacopa monnieri (Linn.)* and *Isodon rugosus (Wall. Ex Benth)*.

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Callus technology can be utilized to produce antioxidants and other metabolites in industrial quantity. Most of the metabolites from plants have been found to have medicinal values or useful to mankind and antioxidant is one of them.

Keywords: Antioxidants; callus technology; explants; medicinal plant; metabolites and plant growth hormones.

1. INTRODUCTION

Antioxidants are molecules that have the ability to inhibit the oxidation of other molecules. Oxidation can be referred to as chemical reaction that transfers hydrogen or electron from substances to an oxidizing agent. Oxidation has the ability to start chain reactions and also produce free radicals but antioxidants inhibit oxidative reactions and also terminate chain reactions by removing the free radicals [1,2]. Antioxidants are often reducing agents like ascorbic acid, thiols or polyphenols [3]. Free radicals are always produced in the body because of oxidative processes which are responsible for many diseases [4]. Even though the human body works in such a way to neutralize these free radicals produced in our body, the kind of food one eats determines how this free radical will be eliminated from the body. It is therefore recommended that food rich in antioxidant will make the body resistant to the free radicals [5]. Improper eating life style of an individual and stress increase the production of free radicals in the body system. These free radicals encourage a chain reaction in human beings which makes other molecules unstable and result in adverse effect of metabolism. Human system is designed in such a way to combat these free radicals with the help of antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalase [6,7]. The recent awareness in the importance of good health and disease free life have cause devotion to medicinal plants with high antioxidant activities [8,9]. Natural antioxidants are gotten from plants and the needs for antioxidants are always increasing so as to prevent the effects of free radicals in humans and natural antioxidants are gotten from plants [10]. Antioxidants from plant as natural source have been used to prevent oxidation, the treatment of several diseases as therapeutics, controlling pathogens or toxin-producing microorganisms in foods [11-14]. Oxidative stress is involved in pathophysiology of many progressive disorders such as neurodegenerative processes, cancer development, cardiovascular diseases [15], cognitive dysfunctions [16-18], diabetes [19], Huntington's disease [20], amyotrophic lateral sclerosis [21], Alzheimer's disease [22,23], Parkinson's disease [20] and other aging-associated diseases [24].

Production of callus from explants is normally carried out to determine the culture conditions required by the explants to grow, to study cells development and to exploit metabolites that are produced [25, 26]. Callus culture has the ability to produce bioactive compounds in large quantity from plants [27] and these bioactive compounds might be absent in the wild types [28]. Successful callus is determined on the basis of secondary metabolites and biomass produced with right plant nutritional media, growth regulator and growth conditions available [29]. Callus culture has been used to produce different classes of compounds whose applications cut across different kind of diseases. Biotechnology has exploited the use of callus culture in the production of many plant derived compounds which antioxidant is not excepted [30,31].

The aim of this review is to study the different methods that different authors have used to produce antioxidant from callus culture of; *Sericostoma pauciflorum, Helicteres angustifolia L, Lepidium sativum L, Randia echinocarpa, Andrographis paniculata Nees, Citrullus colocynthis, Rauwolfia vomitoria Afzel, Decalepis hamiltonii, Bacopa monnieri (Linn.) and Isodon rugosus (Wall. Ex Benth)*.

2. CALLUS CULTURE FOR ANTIOXIDANT

2.1 Antioxidan Culture from *Sericostoma pauciflorum*

Sericostoma pauciflorum Stocks ex. Wight, which is popularly known as "karvas", is a xerophytic plant which is very important in herbal medicine. The Genus Sericostoma has eight species of plants which are distributed throughout the Tropical North West of India and North East of Africa. It is found growing all over coast of

Maharashtra and Saurashtra which is use in making a key drug in Ayurveda named "Krishnavalli" that is used against diabetes, dehydration, acidity and cancer. The plant contains medicinally important secondary metabolites such as pauciflorinyl acetate, β-sitosterol, fridelin, caffeic acid, α-amyrin, β-amyrin, leupeol, pauciflorol acetate and sericostinyl acetate which is used as antioxidant, anticancer, anti-inflammatory and antibacterial [32-35].

The culture media for *S. pauciflorum* was prepared by supplementing MS media with Indole-3-butyric acid, Indole-3-acetic acid and Kinetin at different concentrations of (0.5, 1, 1.5 and 2 mg/L). At six weeks of culture the callus extracts were extracted using petroleum ether, water and methanol. The callus antioxidant of the aqueous extracts were more active where Kn (kinetin) and IBA (indole 3-butyric acid) extracts showed 0.06 mg/mL IC50 value (% inhibition 93.30 and 92.70 respectively at 0.8 mg/mL concentration) with 343 ± 3.34 and 366 ± 6.69 ascorbic acid equivalent antioxidant potentials at 1 mg/mL concentration [36].

2.2 Antioxidan Culture from *Helicteres angustifolia L*

Helicteres angustifolia (Sterculiaceae) is known to be widely distributed in southern China, Japan and Southeast Asia, where it is known as an important medicinal plant. The roots of this plant have been used in places like Chinese or Laos by folk medicine agents for long. Recent photochemical studies have shown that the plant has many bioactive compounds such as flavonoids and phenolics [37], triterpenoids [38], alkaloids [39], polysaccharides [40] and steroids [41]. The most important compounds in this plant are use in promoting health like antioxidant, immunomodulatory, anticancer and antidiabetic [42 43,44].

H. angustifolia callus culture was prepared by using suspension culture was prepared using where MS media was supplemented with 3.0 mg/L NAA, 0.4 mg/L ascorbic acid and 3% (w/v) sucrose was used. Culture was harvested every seven days to check for dry matter. Bioactive assays showed that the extract of callus suspension cultures has strong antioxidant activities compared to the equivalent wild roots [45].

2.3 Antioxidan Culture from *Lepidium sativum L.*

Lepidium sativum L., is a herbaceous medicinal plant which is popularly called garden cress and belongs to the family Brassicaceae and it has height of between 30 to 50 cm. The plant is edible particularly it's seeds which have many health benefits [46-48]. It has Biological activities such as anticancer, antimicrobial, allelopathic and bronchodilator activity from the aqueous extract of the plant [49,50]. It also has natural anti-oxidant, carotenoid and vitamin E which protect the oil from rancidity. Seven imidazole alkaloids, in which five lepidine (C, E, B, F and D) are dimeric and there are two monomeric semi lepidinoside A and B alkaloid which were studied in seeds [51]. Photochemical analysis of the plant showed the existence of important polyphenolics (phenolic acids and flavonoids) constituents which are sinapic acid and its derivative, quercetin, kaempferol, caffeic acid, pcoumaric acid, and other phenolic compounds like glucosinolates that has antioxidant potential [52,53].

L. sativum callus culture was prepared by using MS media which was supplemented with different concentrations of thidiazuronn (TDZ) and α-naphthalene acetic acid alone and in combination was used as the media. Monochromatic lights with different source were employed as elicitor on explant derived callus which are White Light (24 h, wavelength 400–700 nm), Green Light (24 h, wavelength 510 nm), Blue Light (24 h, wavelength 460 nm), Yellow Light (24 h, wavelength 570 nm), Red Light (24 h, wavelength 660 nm), Dark (24 h), and photoperiod cycle (16/8 h light/dark) where callus was harvested after 28 days of inoculation. White light grown cultures gave optimum antioxidant activity (629.78 µM), followed by blue light (576.41 µM) and dark (552.36 µM) respectively [54].

2.4 Antioxidan Culture from *Randia echinocarpa*

Randia echinocarpa Moc. & Sessé ex DC. (Rubiaceae) is found along the Pacific coast of Mexico and the plant is popularly known as "papache" in the state of Sinaloa, which has edible pulp and it has been used in traditional medicine for the treatment of malaria, diabetes and cancer, as well as for gastrointestinal infections, lung, kidney andcirculatory [55,56]. Santos-Cervantes et al. (2007) [57] reported the

antimutagenic and antioxidant activities of the fruit while Cano-Campos et al. (2011) [58] also reported that methanol extracts of the fruit has strong antimutagenic activity, which is as a result of linoleic acid, palmitic acid, and β-sitosterol content of the plant. In a current research, Montes-Avila et al. (2018) [59] reported that purified melanins from the R. echinocarpa fruit had protective effect against H2O2 stress on Saccharomyces (antioxidant activity) which can be used in treating degenerative diseases.

Randia echinocarpa callus culture was prepared by using MS Media which was supplemented with benzyl aminopurine (0.2, 0.6, and 1 mg/L) and indole acetic acid (1 and 2 mg/L). Antioxidant activity of the methanol extract obtained from calli of hypocotyls and cotyledons, of R. echinocarpa. The methanol extract showed the highest antioxidant activities in both DPPH (345.5 µmol TE per 100 g d.w.) and ABTS (1166.4 µmol TE per 100 g d.w.) assays [60].

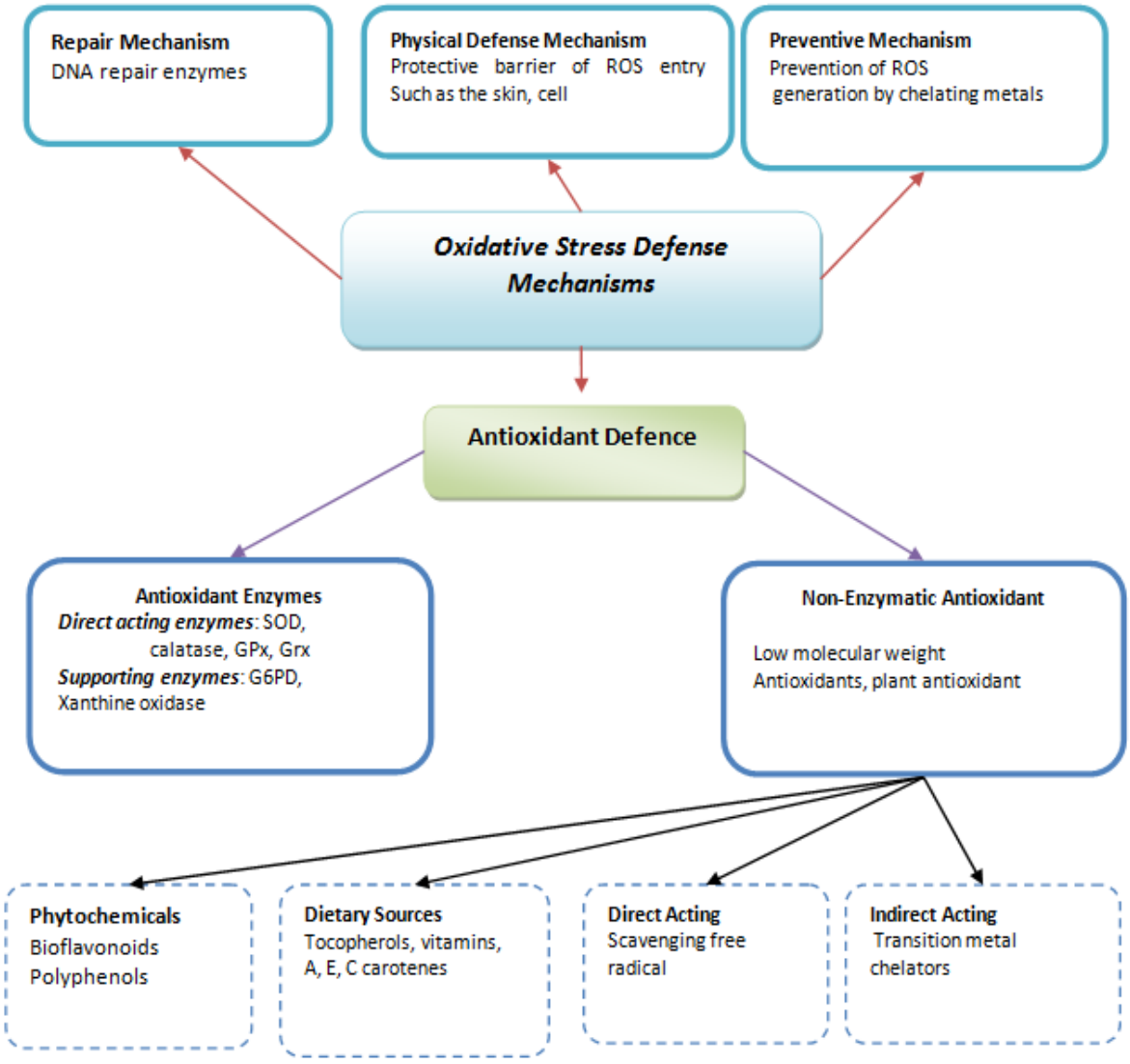


Fig. 1. Antioxidant defence mode of action

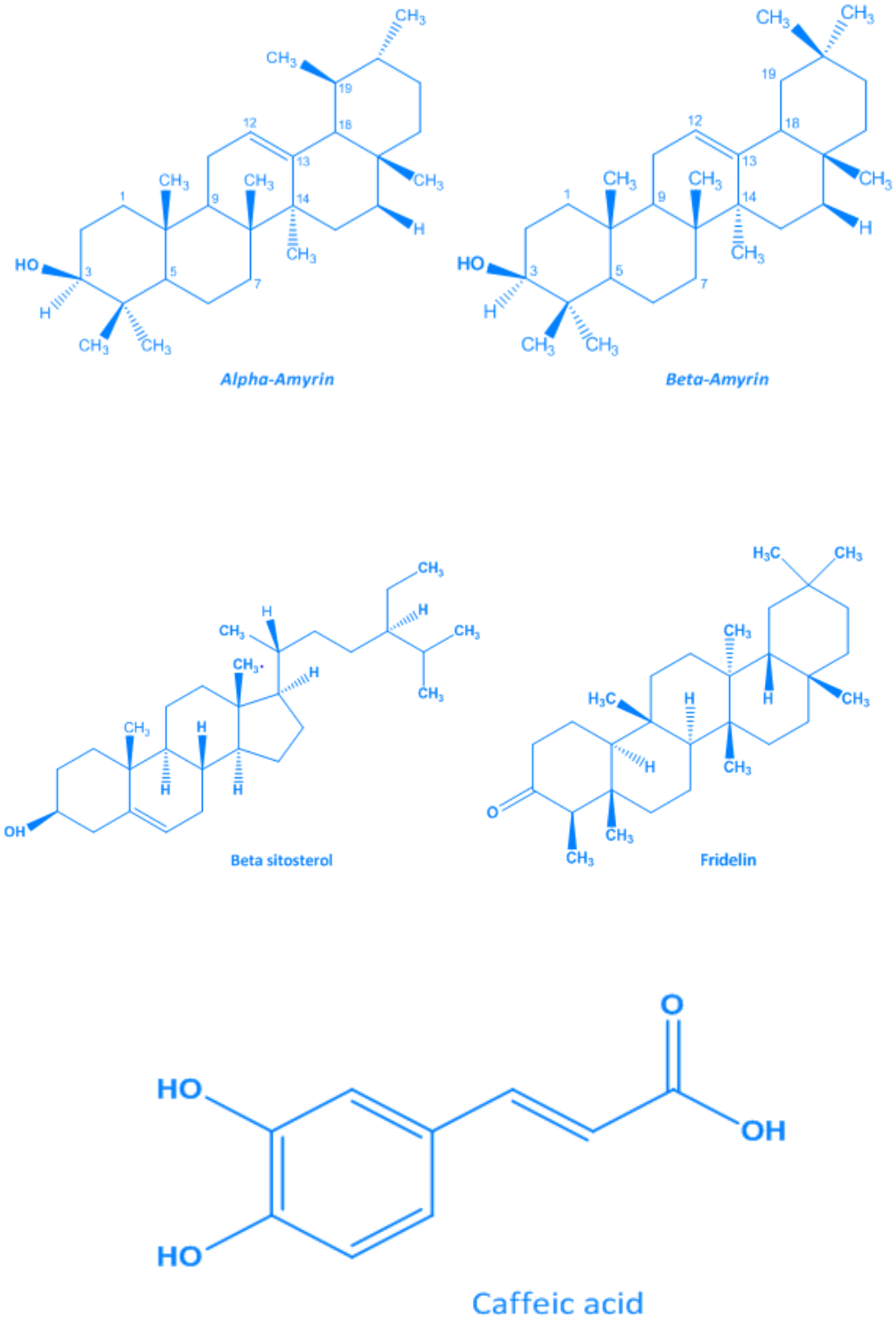


Fig. 2. Chemical Structure of some Phytochemicals present in *Sericostoma pauciflorum*

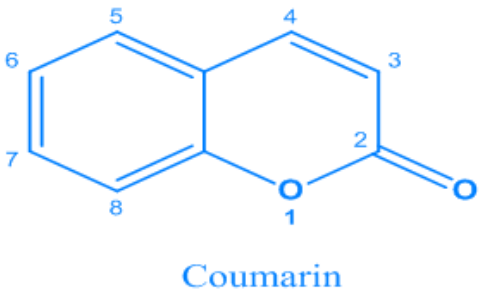


Fig. 3. Chemical structure of coumarin

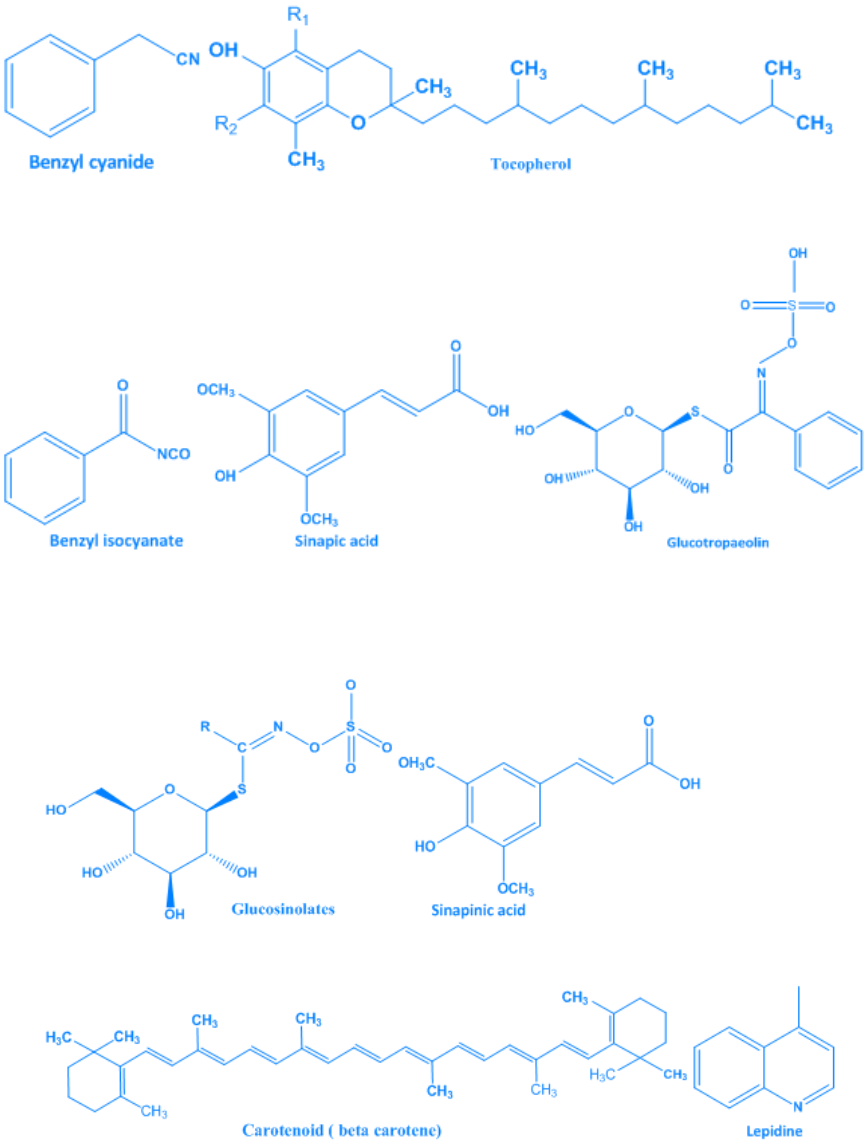


Fig. 4. Chemical Structure of some Phytochemicals present in *Lepidium sativum* Linn

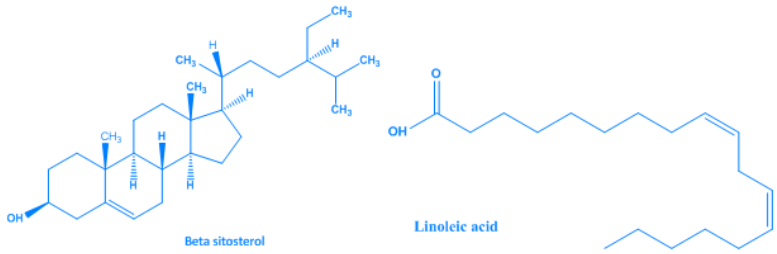


Fig. 5. Chemical Structure of some Phytochemicals present in *Randia echinocarpa*

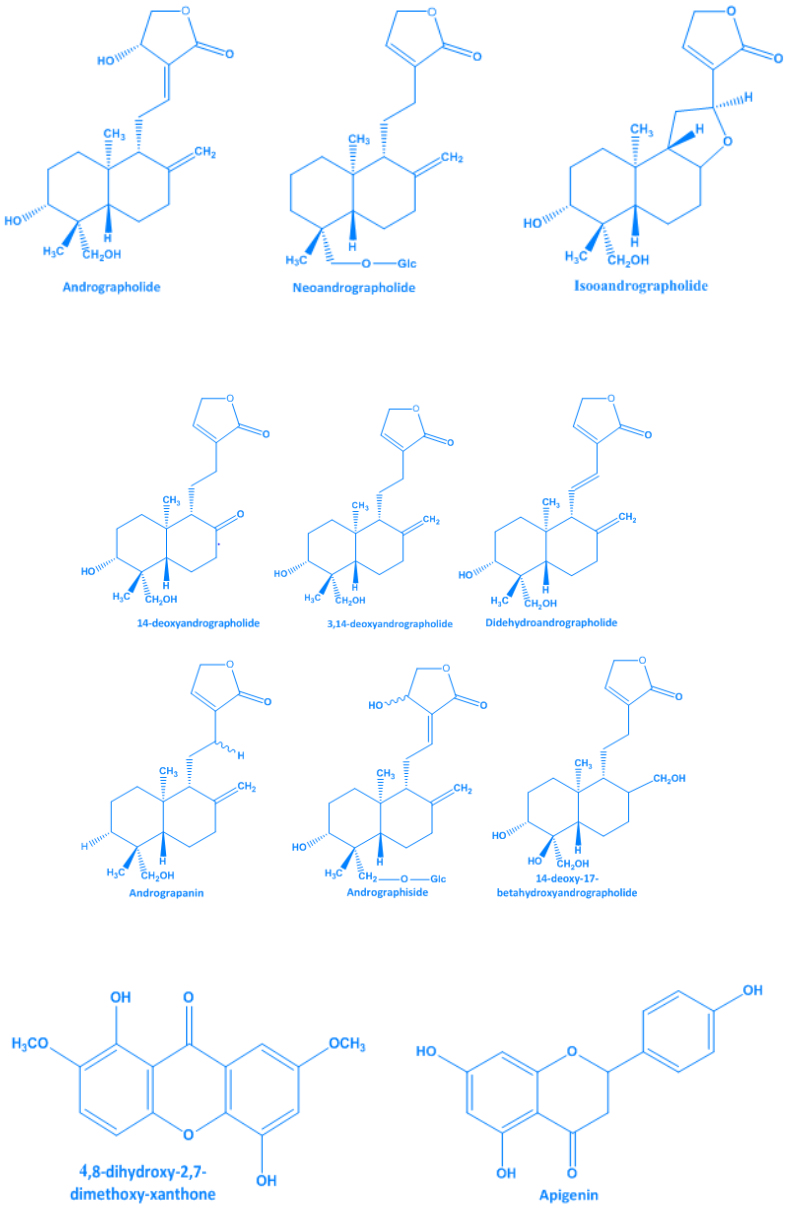


Fig. 6. Major phytochemical constituents from *Andrographis paniculata*

2.5 Antioxidan Culture from *Andrographis paniculata Nees*

The genus Andrographis (Acanthaceae), is made up of about 40 species, which is normally distributed in tropical Asia and southern India. *Andrographis paniculata Nees* is usually called King of bitters or Creat in English because of its bitter taste, Kalmegh in India, and Senshinren in Japan. It is a well known ethno-medicinal herb used for treating inflammation, infection, fever, snake bite, cold, diarrhea, kidney diseases, dysentery, jaundice, and anti-oxidant agent [61]. Its photochemical analysis showed the presence of andrographolides and flavonoids [62], different parts of this plant have been shown to have numerous therapeutic compounds like anti-cancer, antidiabetes [63,64], anti-inflammatory [65], anti-typhoid, antiviral, anti-malarial, antipyretic, hepatoprotective [66], anti-human immunodeficiency virus and immunostimulatory activity [67]. The anti-oxidant activity of the plant is related to the total phenolic content of the extract, and there is a correlation between the content of antioxidant activities and phenolic compounds [68].

A. paniculata Nees callus culture was prepared by using MS medium which was supplemented with different concentrations of indole acetic acid, 2, 4-D and α-naphthalene acetic acid. Anti-oxidant activities of andrographolide and echiodinin of A. paniculata extract was determined. Echiodinin showed relatively higher total antioxidant activity in a given tested concentration as compared to andrographolide [5].

2.6 Antioxidan Culture from *Citrullus colocynthis*

Citrullus colocynthis is a medicinal plant species of Cucurbitaceae family which is commonly known as bitter melon or Colocynth. It is widespread in different parts of South Eastern Desert of Egypt, which usually grows fast in sandy soils [69] .It is known to secrete different secondary metabolites such as flavonoids, cucurbitacin, terpenoids caffeic acid derivatives, flavonoid glycosides and cucurbitacin glucosides [70,71]. Colocynth fruit extracts have antibacterial and antifungal prevalent in dermatology [72]. In the callus culture of colocynth higher content of total cucurbitacin and cucurbitacin-E was found [73].

C. colocynthis callus culture was prepared by using MS medium which was supplemented with different combinations of Kinetin and 2,4-dichlorophenoxyacetic acid (2,4-D) as well as NAA and BA in different concentrations. Seedlings of C. colocynthis were used as source of explants for initiation of callus cultures from stems, roots and leaves. Leaf-derived calli was cultured on MS medium supplemented with 2.0 mg/L 2,4-D + 1.0 mg/L kinetin (MD1) gave the highest DPPH radical scavenging activity. The highest percentage of H2O2 scavenging activity was gotten from leaf explant-derived calli growing on MS + 2.0 2, 4-D + 1.0 kinetin. The leaf-derived calli growing on MS + 6.0 2, 4-D + 2.0 KIN gave the highest ferric reducing power (22.3 µg/g d.w.), compared to the activities of leaves, stems, and roots of in vitro grown seedlings (3.28, 12.9 and 2.85 µg/g d.w.), which were used as controls. Therefore, MS media supplemented with different combinations of 2,4-D and kinetin gave higher antioxidant activities than MS media supplemented with NAA and BA [74].

2.7 Antioxidan Culture from *Rauwolfia vomitoria Afzel*

*Rauwolfia vomitoria Afze*l, a tropical shrub found in the family of Apocynaceae, is commonly used as ethnomedicines especially in West African sub-region. It is commonly referred to as Poison devil's pepper, swizzle stick (English), and African serpent wood or African snakeroot. The plant occurs mainly in bush vegetation, secondary vegetation (fallow land), and gallery forest and along roadsides. It is a plant with medicinal valued for its antipsychotic property and used in various herbal preparations for the treatment of insomnia, malaria, hypertension, nervous disorder, jaundice, scabies and diarrhea [75, 76]. The parts mostly used are the root and leaves [77, 78]. It is reported to possess important multiple therapeutic properties viz. hepatoprotective activity [78]; hypoglycemic activity [79]. It has more than 50 active indole alkaloids, each possessing remarkable pharmacological activities [80].

*R. vomitoria Afze*l callus culture was prepared by using MS medium which was supplemented different concentrations of 6-benzyl amino purine (BAP), α-Naphthalene acetic acid (NAA) and 2,4-Dichlorophenoxy acetic acid (2, 4-D). The inoculated explants were maintained for a period of six weeks where both the leaf and root explants were extracted using methanol. The highest antioxidant activity was seen from the

root extract of the wild plant and Leaf-derived callus had the least antioxidant effect [81].

2.8 Antioxidan Culture from *Decalepis hamiltonii*

Decalepis hamiltonii Wight & Arn. commonly called as swallow root, is a monogeneric medicinal shrub belonging to the family Apocynaceae, is a plant with history of traditional use such as the ethnomedicinal species of Decalepis are of particular interest as herbal medicines and a source for novel bioactive compounds [82]. Apocynaceae known for its antioxidant property [83]. The young roots contain about 92% fleshy matter and 8% woody core. The roots of this plant are highly aromatic and contain metabolites like aldehydes, alcohols, ketones, sterols and triterpenes, of which 2-hydroxy-4-methoxybenzaldehyde is the principle component [84,85]. The root extract exhibits antibacterial, antifungal, anti-inflammatory, antipyretic, chemoprotective, hepatoprotective and most importantly, antioxidant properties [86]. When consumed, it cools the system, gives good appetite and also acts as a blood purifier [87].The root extract also acts as neuroprotectant [88] and attenuates the age-related decline in cognitive ability, in addition to ameliorative effect on the memory of the offspring in Drosophila [89].

D. hamiltonii callus culture was prepared by supplementing MS media with 0.4 mg/L 2,4–D, 1 mg/L 2,4–D and 2 mg/L 2,4–D. Another set was supplement NAA 2mg/l + BAP 0.5mg/l, NAA 1mg/l + BAP 0.5mg/l, and NAA 0.5mg/l + BAP 1mg/l where both (root and callus) extracts were extracted using methanol. The total antioxidant capacity of root extracts gave higher degree of antioxidant capacity than that of the callus extract [90].

2.9 Antioxidan Culture from *Bacopa monnieri (Linn.)*

Bacopa monnieri, is popularly known as water hyssop and is widely used by some people as a memory enhancer. The plant also protects the brain against neurodegenerative disorders such as Alzheimer's and Parkinson's [91]. Phenolic compounds like caffeic acid and chlorogenic acid are found in the extract of the plant [92]. It was also found out that the extract of B. monnieri decreases the free radical accumulation in the brain which promotes defense against oxidative stress [93]. γ-irradiation promotes the plant's total phenolic contents and antioxidant activity [93, 95].

Bacopa monnieri callus culture was prepared with MS media which was supplemented with two different hormone concentrations; 1.5 mg/L NAA + 0.5 mg/L BAP and 1.5 mg/L 2,4-D + 0.5 mg/L BAP individually, was used for callus culture induction. The calli were irradiated for 2, 3, 4 and 5 minutes separately at a distance of 30 cm. The antioxidant potential was greatly enhanced in the treated calli and significantly increased when compared to the control. The results showed that UV-C irradiation is a useful method in enhancing the accumulation of antioxidant or phytochemicals in the callus cultures of *B. monnieri* [96].

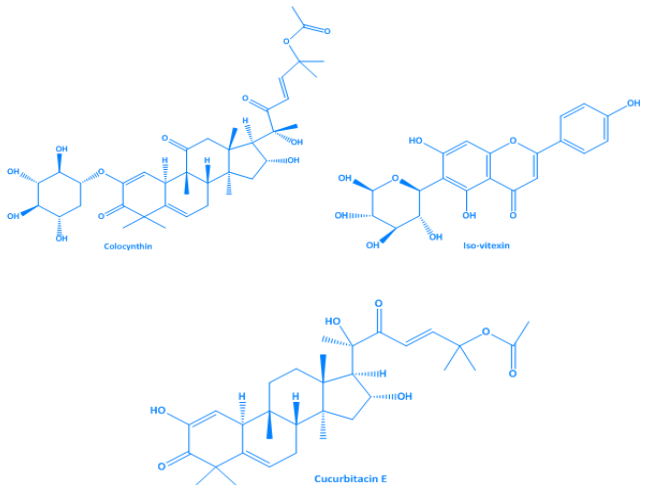


Fig. 7. Phytochemical constituents of *Citrullus colocynthis* (Colocythin, Isovitexin and Cucurbitacin E)

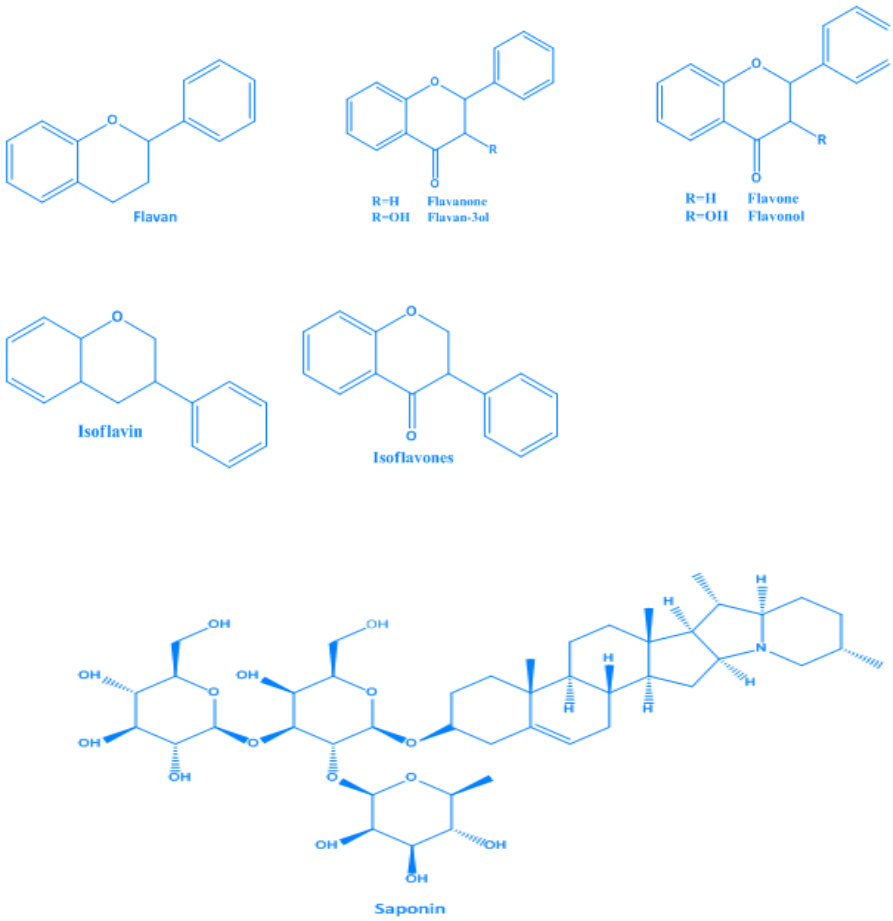


Fig. 8. Phytochemical constituents of *Rauwolfia vomitoria*

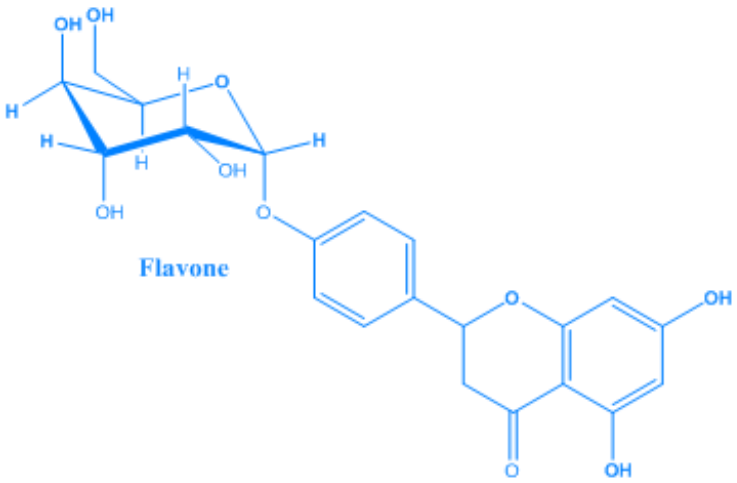


Fig. 9. Phytochemical constituent present in Rauwolfia vomitoria Afzel

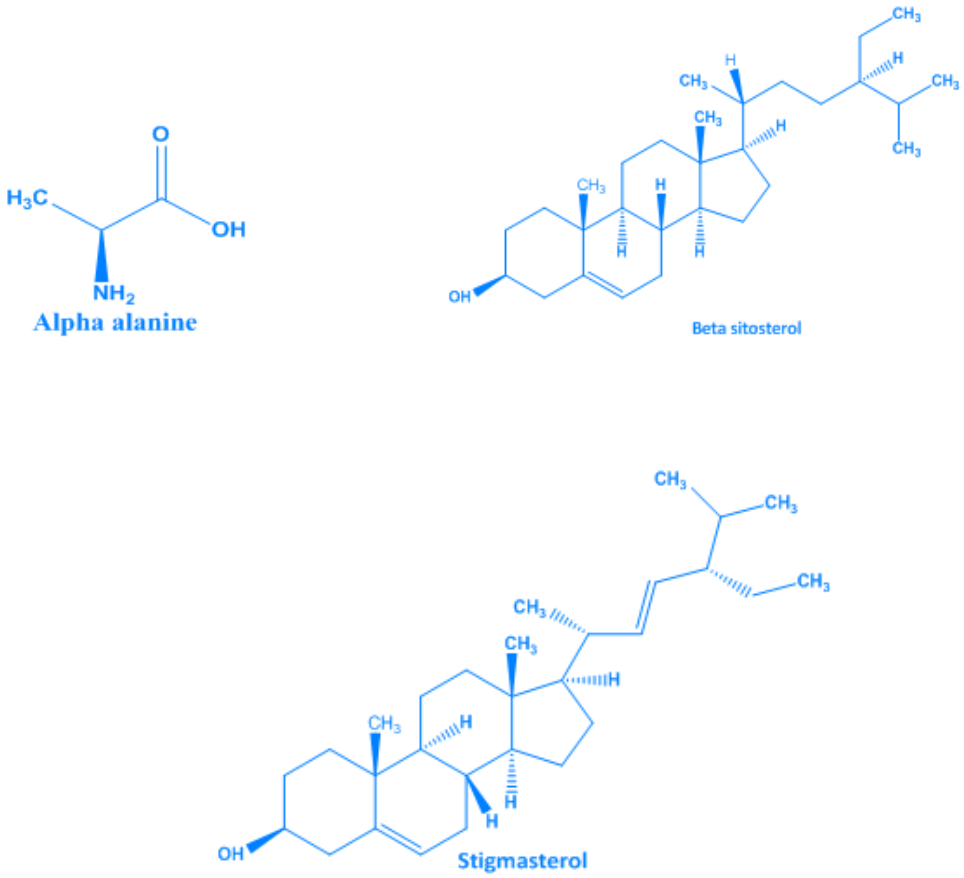
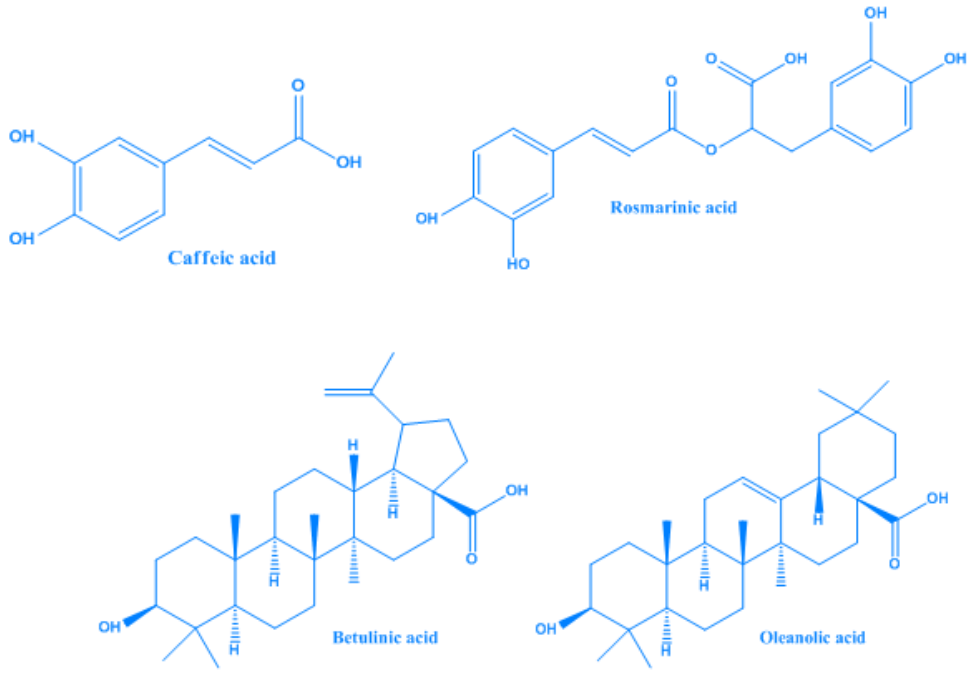


Fig. 10. Phytochemical constituent present in *Bacopa monnieri* (Linn.)



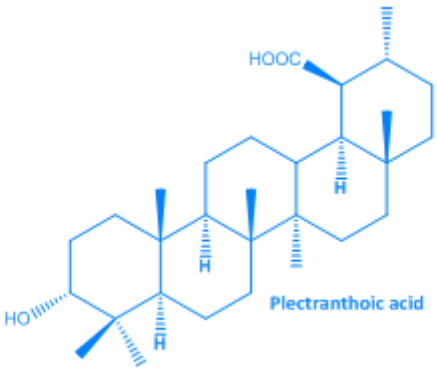


Fig. 11. Phytochemical constituent present in *Isodon rugosus*

Table 1. Antioxidant production from different plants calluses

S/NO	Name of Plant	Explants	Method of Extraction	Plants Hormones Used	References
1	*Sericostoma pauciflorum*	Stem	Petroleum ether, Methanol and Water	MS supplemented with Indole-3-acetic acid, Indole-3-butyric acid IBA and Kinetin	[36]
2	*Helicteres angustifolia L*	Callus from the leaves	Ethanol	MS supplemented with Indole-3-acetic acid and ascorbic acid	[45]
3	*Lepidium sativum L.*	Leaf and stem	Methanol	MS supplemented with thidiazuron, α-naphthalene acetic acid and other hormaones.	[54]
4	*Randia echinocarpa*	cotyledon and hypocotyl explants	methanol	MS supplemented with Benzyl aminopurine and indole-3-acetic acid	[60]
5	*Andrographis paniculata Nees*	Leaves	Acetone and Methanol	MS supplemented with NAA, 2,4-D and IAA	[5]
6	*Citrullus colocynthis*	stems, leaves and roots	Methanol	MS supplemented with 2,4-D with kinetin and Benzyladenine with α-naphthaleneacetic acid	[74]
7	*Rauwolfia vomitoria Afzel*	Leaf and Root	Methanol	MS supplemented with α-naphthaleneacetic acid, 2, (2,4- D) and BAP	[81]
8	*Decalepis hamiltonii*	Root and Callus	Methanol	MS supplemented with 2,4-D, NAA and BAP	[90]
9	*Bacopa monnieri* (Linn.)	Leaf	Water	MS supplemented with 2, 4-D, Naphthalene Acetic Acid, benzylaminopurine, Kinetin.	[96]
10	*Isodon rugosus* (Wall. Ex Benth.)	Stem And Leaf	Methanol	MS supplemented with Benzyl aminopurine, α-naphthaleneacetic acid and thidiazuron.	[107]

2.10 Antioxidan Culture from *Isodon rugosus (Wall. ex Benth.)*

Isodon rugosus (Wall. ex Benth.) is an aromatic shrub which stems are erect with the quadrangular branches, broadly ovate shape that has green color, the leaves are opposite; leaf blade consist of small stellate dendroid hairs. Its inflorescence is Cymose, Nutlets fruit is an oblong shape with dark brown color, each flower is white, spotted pink or violet, bilabiate form. This plant is rich in bioactive compounds that have cosmetics ingredient, aromatic plant containing essential oils [97- 100]. It also contains caffeic acid phenolic and pentacyclic triterpenes derivatives have been also and pentacyclic triterpenoids plectranthoic acid, betunilic acid, oleanolic acid and other phenolic compounds [101] .It has medicinal values such as hypertension, dementia, toothache, cancer, fever, rheumatism, antimicrobial, hypoglycemic, phytotoxic, antidiarrheal, lipoxygenase inhibitory, anticholinesterase, bronchodilator, and anthelmintic [96,102-106].

I. rugosus was prepared with MS media which was prepared by supplementing MS media with thidiazuron (TDZ) used alone or in conjunction with NAA or BAP where TDZ (1.0 mg/L, 2.0 mg/L and 3.0 mg/L) and 1.0 mg/L TDZ + NAA (1.0 mg/L, 2.0 mg/L and 3.0 mg/L) give the highest callus induction (95–100%) compared to other combinations. All the callus extracts exhibited marked antioxidant and chelation activities. Stem-derived calli grown on 1.0 mg/L TDZ and 3.0 mg/L NAA showed highest antioxidant activities for all of the assays. The leaf-derived calli grown on 1.0 mg/L TDZ give the lowest antioxidant activities with values. The stem-derived callus extracts gave the higher antioxidant activities than the callus initiated from leaf explants [107].

3. DISCUSSION

Since early times, plants derivatives contribute to a large extent for herbal and health medicine, which is gotten from compounds found in the plants. The search for new compounds is always increasing [108-112] and using callus technology offers better options [30]. The authors mentioned above used different techniques in callus culture to enhance the production of antioxidant. Meenashree *et al.,* (2018) report the use of irradiation with UV-C light (254 nm) for 2, 3, 4 and 5 minutes to enhance the production of antioxidant in *Bacopa monnieri* (Linn.), where the treated calli had more antioxidant than the untreated ones [95]. Valenzuela-Atondo *et al.,* (2020) examined the effect of auxin and cytokinin ratio on calli induction from cotyledon and hypocotyl explants in *Randia echinocarpa*, where calli from hypocotyls give a higher antioxidant activity [60]. El-Baz *et al.*, 2010 [107] report the manipulation of different concentrations and combinations of plant growth regulators in *Citrullus colocynthis* to produce antioxidant, where MS media was supplemented with different combinations of 2,4-D and KIN yields higher antioxidant activities. Arifullah *et al.,* (2013) [5] also reported the manipulation of MS medium with different concentrations of auxins and active constituents (andrographolide and echiodinin) of *Andrographis paniculata*, where echiodinin showed relatively higher total antioxidant activity. Jain *et al.,* (2012) [36] stated the effects of different growth hormone on the production of antioxidant in *Sericostoma pauciflorum* where dried calli was extracted successively in methanol, petroleum ether and water and the antioxidant potentials of all the aqueous extracts were more active. Ullaha *et al.,* (2019) [54]examined the influence of different monochromatic lights in controlled condition for the production of antioxidant in *Lepidium sativum* where callus cultures of this plant under white light for 24 hours gave highest antioxidant activities. Yang *et al.,* (2019) [45] look into the use of callus suspension cultures of *Helicteres angustifolia* L to produce antioxidant, where the callus suspension culture proven to be an effective alternative for antioxidant. Abbasi *et al.,* (2019) [106] also reported the callus induction from stem and leaf explants of *Isodon rugosus* (Wall. Ex Benth) under different plant growth regulators for the production of antioxidant where stem-derived calli was grown on 1.0 mg/L TDZ + 3.0 mg/L NAA) displayed highest antioxidant activities. Sonibare and Akpan (2016) [80] examined the investigation of antioxidant activity of the wild plant and the leaf-derived callus of *Rauwolfia vomitoria* Afzel where the highest antioxidant activity was obtained from the root extract of the wild plant. Umesh (2014) [89] reported the investigation of the antioxidant potential of tuberous root and callus from *Decalepis hamiltonii*, where root extracts showed higher degree antioxidant capacity.

Many other authors have also reported the use of callus culture to produce antioxidant from explants. Hossain and Uddin (2019) [113] investigated the antioxidant properties of leaf explants regenerated from shoot tip, broccoli

root, and leaf cutting, where the leaf extracts showed the highest antioxidant activity. Vats (2012) [114] also reported MS medium supplemented with various concentrations of auxins and cytokinins of *Vigna unguiculata* (L.) Walp. and Methanolic extract of callus which was successively partitioned with chloroform, n-hexane, and ethyl acetate where maximum antioxidant activity was observed in ethyl acetate fraction. Singh and Chaturvedi 2018 [115] investigated the antioxidant activity of different parts (Rhizome, fruit, leaf and callus) of *Rheum emodi* where all the parts of *R. emodi* possessed significant antioxidant activity but recommended that not only the rhizomes, but aerial parts as well as calli can also be utilised for antioxidants. Sokmen *et. al.,* 2004 [116] evaluated the antioxidant from Origanum acutidens and using methanol extracts and butylated hydroxytoluene, where methanol extracts obtained from herbal parts showed better antioxidative activity. There are also many other report on the use of callus to produce antioxidant [117-122].

Callus technology is the techniques of producing callus in the presence of explants; these techniques involves the collection of viable explants, surface sterilization, plant hormones combinations, proper environmental conditions, inoculation of an explants, production of callus and/or production of useful metabolites. Many researchers use callus technology to discover the type of metabolites callus can produce and at what quantity. Callus technology has been used to produce many compounds such as antioxidant, anti-aging, phytochemicals, antibacterial, antifungal, antiviral, antitumor, cosmetic extracts and pesticides. From this review, one can use different callus technology such as exposing callus to UV to examine the amount of antioxidant produced, checking different parts of a particular plant for the amount of antioxidant present, using different hormonal combinations to determine the amount of antioxidant that will be produced, different types of extraction solvent that give high amount of antioxidant, and using culture media (suspension culture or callus) to determine the quantity of antioxidant that will be produced.

4. CONCLUSION

The quantity of antioxidant production from callus technology (culture) is determined by the kinds of plant hormonal combinations, type of culture used (suspension or callus), the plant parts used as explants, the species of the plant, method of extraction and UV light used during callus formation. Callus technology is very promising for the discovery and also greater quantity of metabolites from plant. Callus technology is also important due to the availability of callus to produce required metabolites all year round compared to plant parts which its availability is seasonal.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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