

Secondary Metabolites and their Antioxidant Activities: An Overview

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Received: July 14, 2020; **Published:** December 31, 2020

Abstract

Free radicals/reactive oxygen species (ROSs)/reactive nitrogen species (RNSs) are produced by cells through endogenous reactions, various physicochemical events or pathological issues. Antioxidants (AOX) are compounds scavenge or regulate these radicals. The balance between these molecules and AOXs is a pre-requisite for healthy physiological functions of the body. If free radicals/ROS/RNS over expresses in the system results into oxidative stress and other life style disorders i.e., oxidize lipids, proteins, nucleic acids and trigger a number of human diseases. Therefore, inclusion of antioxidants in food can assist in balancing this sort of oxidative bursts. Chemical antioxidants like butylated hydroxy anisole, butylated hydroxy toluene, propyl gallate and tert-butyl hydroquinone were employed in food resulted in to dangerous ill effects. Thus, the search for plant based biomolecules with antioxidative activity has been explored in recent times. The present overview provides an account of plants employed by the local people as antioxidants or functional foods or stress reliever in alleviating human diseases. The secondary metabolites analyzed in the present study as antioxidants include polyphenolics, carotenoids, alkaloids, terpenoids and flavonoids. These molecules function either as free radical scavengers or function by retarding chain initiation or involved in repair of damaged biomolecules. The output of the study will assist in providing knowledge regarding appropriate applications of the natural bioactive components in the regular diet as nutraceuticals.

Keywords: Antioxidant; Free Radicals; Oxidative Stress; Secondary Metabolites; Ethnic People

Introduction

Reactive radicals such as reactive oxygen species (ROSs) and reactive nitrogen species (RNS), are produced inside the living systems through multiple metabolic pathways. The cell system is in built with antioxidants that safe guard the cells from oxidative burst caused by these free radicals. Antioxidants (AOX) like glutathione, thioredoxin and AOX enzymes (superoxide dismutase, glutathione peroxidase, catalase regulates the oxidative stress and protect lipids, proteins, and nucleic acids. Similarly, tocopherols, ascorbate, carotenoids, flavonoids, amino acids are also natural AOXs present in the vegetables, fruits and leaves. There is a high demand for such foods balanced with AOXs and probiotics that may vitalize the human health. AOXs had a growing demand in pharmaceutical field owing to their protective roles in scavenging ROS/RNS against oxidative stress induced pathological event. Exploration of AOX features of herbals requires proper protocols, which address the mode of AOX activity and its kinetics of the reactions. Many reports were correlated about the AOX potentialities of plant based secondary metabolites using wide protocols with human health. Protocols like inhibited autoxidation connected

with termination-enhancing AOXs, for chain-breaking AOXs, and preventive AOXs are unique. The easy protocol is *in vitro* determination of AOX power of food items. Some of the methods are 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate), 2,2-diphenyl-1-picrylhydrazyl radical scavenging, Fe^{3+} - Fe^{2+} transformation method, ferric reducing antioxidant power protocol, cupric ions reducing power, Folin-Ciocalteu reducing power, peroxy radical ($\text{ROO}\cdot$), superoxide radical anion, hydrogen peroxide, hydroxyl radical, singlet oxygen ($^1\text{O}_2$), nitric oxide radical ($\text{NO}\cdot$) scavenging and chemiluminescence assay. Many phytochemicals from indigenous plants have been found to possess antioxidant activity under *in vivo* and *in vitro* assays. In fact, only a few species have been validated therapeutically useful under *in vivo* conditions due to their interference with physiological processes like absorption, distribution, metabolism, storage and excretion. However, the phytochemicals are being screened only in terms of *in vitro* antioxidant activity and the data are then directly employed to their therapeutic usefulness. In this juncture, the overview of the present study refers selected ethnic plants used by the native people and their applications.

Materials and Methods

The plants were selected based on the traditional usage by the native people or tribes of the locality. The algal species includes *Gracilaria dura*, *Kappaphycus alvarezii* and *Hypnea musciformis*; bryophytes: *Plagiochila beddomei*, *Leucobryum bowringii*, *Octoblepharum albidum*, *Marchantia polymorpha*, *Marchantia linearis*, *Pallavicinia lyellii*, *Thuidium tamariscellum*; angiosperms: *Solanum aculeatissimum*, *Solanum mauritianum*, *Artemisia japonica*, *A. nilagirica*, *Premna serratifolia*, *Orthosiphon aristatus*, *Pouteria campechian*, *Pogostemon benghalensis*, *P. cablin*, *Thottea siliquosa*, *Tylophora Subramanii*, *Begonia cultivars*, *Osbeckia* species *Clerodendron infortunatum*, *Tectona grandis* and Wild Balsam Species. Protocols used were 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate), 2,2-diphenyl-1-picrylhydrazyl radical scavenging, Fe^{3+} - Fe^{2+} transformation method, ferric reducing antioxidant power protocol, cupric ions reducing power, Folin-Ciocalteu reducing power, peroxy radical ($\text{ROO}\cdot$), superoxide radical anion, hydrogen peroxide, hydroxyl radical, singlet oxygen ($^1\text{O}_2$) scavenging assay.

Results and Discussion

Sumayya., *et al.* [1] explored the local usage of seaweeds as sources of ailments for curing many human disorders along the coastal belts of Kerala and established that they are proven sources of biologically active metabolites. Many of the active principles produced by marine seaweeds were used in traditional and complementary medicine. Hence, a preliminary phytochemical survey of the seaweeds will be needed to reveal its secondary metabolite constituents and their by channeling towards its therapeutic values. In this study, the methanol extract of *Gracilaria dura* was subjected to phytochemical analysis to know the secondary metabolites present in the extract. The crude extract was purified by column chromatography. The resultant fraction was subject to GC-MS analysis revealed a pool of terpenoids. Four major terpenoids such as dodecanal, hexadecane, tetradecanal, heptadecane and 6, 4, 10-trimethyl-2-pentadecanone (sesquiterpenoid) were noticed. The purified terpenoid extract showed remarkable antioxidant activity in terms of scavenging hydroxyl radicals in a concentration dependent manner. The extract exhibited remarkable profile on the FRAP assay and strong DPPH scavenging potential. The IC_{50} values were significant and comparable with the synthetic antioxidants like ascorbate and quercetin. The obtained results suggest the possible use of red algae, *G. dura* as a good candidate in terms of functional food supplement and also in combating carcinogenesis and inflammatory disorders. Thus, *G. dura* may be considered for future application in medicine, food and cosmetic industries [2].

Similarly, the edible marine algae *Kappaphycus alvarezii* and *Hypnea musciformis*, were evaluated for their phytochemicals followed by its quantification, purification of terpenoids and evaluation of their *in vitro* antioxidant activity in terms of ABTS, FRAP reducing power, superoxide, hydroxyl radical scavenging and hydrogen peroxide radical assay, the metal chelating activities. Qualitative analysis revealed the phytochemicals such as carbohydrates, proteins, alkaloids, glycosides, flavonoids, steroids, saponins, phlobatannins and polyphenolics. The quantified data of *K. alvarezii* were carbohydrate - 38.28 mg/g, protein - 1.06 mg/g and fat - 1.02 mg /g of dry wt., whereas *H. musciformis* revealed 18.02, 1.2 and 0.934 mg/g of dry wt. respectively. Polyphenol forms the major secondary metabolites especially

the terpenoids. The column chromatography purified terpenoids composition from *H. musciformis* and *K. alvarezii* were fractionated by GC-MS analysis. They showed remarkable antioxidant activities in terms of scavenging hydroxyl radicals in a concentration dependent manner. Further, the extract exhibited strong inhibition on the H₂O₂ and DPPH scavenging assay. The IC₅₀ values were significant and comparable with ascorbate and quercetin. Thus, the selected seaweeds may be included in daily dietaries to combat energy malnutrition and micronutrient deficiencies [3].

Remesh and Manju [4] documented the ethnobryological usage of bryophytes by the ethnic tribes from Western Ghats, India. In this scenario following bryophytes were evaluated scientifically for their importance.

Methanol extract of *Plagiochila beddomei* the liverwort was evaluated in terms of antioxidant and antimicrobial activities. Total phenolics was fractionated by high performance liquid chromatography showed the phenolic acids such as coumaric, ferulic, gallic, caffeic, protocatechol, cinnamic, sinapate, chlorogenate and hydroxyl benzoate. Methanolic extract displayed broad spectrum of antimicrobial activity against various bacteria such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus*, *B. subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and fungi like *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *Aspergillus niger*, *A. flavus*, *A. terreus* and *Mucor indicus*. Poor survivorship curve of bacteria and inhibition of fungal spore germination substantiated the antimicrobial power of the extract. *P. beddomei* displayed significant antioxidant activity in terms of 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging ability, ferric-reducing antioxidant power, ferric thiocyanate assay and hydroxyl radical-scavenging activities. Phenols and flavonoids were noticed in high levels. Total phenol content was showed the highest correlation with FRAP assay (R² 0.966). MTT assay also revealed the non-toxic nature of the extract [5].

In addition, Manoj, *et al.* [6] screened *Leucobryum bowringii* and *Octoblepharum albidum*, the bryophytes of Western Ghats in terms of total flavonoids, phenolic content, its fractionation by RP-HPLC and HPLC-PAD. Antioxidant potentialities were examined using DPPH, Ferric Reducing Antioxidant Power, Hydroxyl radical assay, FTC assay and DNA nick assay. Phytochemical study revealed the presence of phenols, saponins, flavonoids, glycosides and tannins. Significant antioxidant potentialities in terms of FRAP, DPPH, hydroxyl radical scavenging and FT assay. Phenols and flavonoids showed many phenolic acids and flavonoids such as quercetin, kaempferol and rutin analogues. Significant linear correlation was observed between total phenolic content and FRAP assay. Reduction in the formation of nicked DNA and simultaneously with increased native form of DNA. Thus, it can be summarized that the selected bryophyte extracts could be an important source of phenolic compounds with high antioxidant capacity comparable with red wine or beverages like tea.

Currently, different therapeutic strategies have been designed for the prevention and treatment of reactive oxygen species mediated diseases, with special emphasis on antioxidant therapy, that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. Among the different phytochemicals, phenolic compounds are established free radical scavengers and antioxidants common among plants; they possess many biological effects, mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals. Present investigation on *Marchantia polymorpha* a bryophyte has been an attempt to screen the phytochemicals and their potential roles in antioxidant potentialities. To complete the objectives, several parameters such as solvent extracts using nonpolar to polar, qualitative analysis of the secondary metabolites, estimation of total phenolic content and its fractionation by RP-HPLC. Antioxidant potentialities were examined with aqueous and ethanolic extracts using DPPH*, FRAP, Hydroxyl radical assay, FTC assay and DNA nick assay. The phytochemical study revealed the presence of phenols, saponins, flavonoids, glycosides and tannins. Significant antioxidant potentialities in terms of FRAP, DPPH, hydroxyl radical scavenging and FT assay. Phenols showed the peaks of many phenolic acids such as gallate, vanilate, chlorogenate, cinnamate, protocatechol, coumarate, ferulate, sinapic, caffeate and hydroxyl benzoate. Significant linear correlation was observed between total phenolic content and FRAP assay. Reduction in the formation of nicked DNA and increased native form of DNA. Aqueous and ethanolic extracts of *Marchantia polymorpha* could be a source of polyphenolic compounds with high antioxidant capacity comparable with standard ascorbate and butylated hydroxytoluene [7].

Remya Krishnan and Murugan [8] screened the phytochemicals and evaluated the antioxidant potentials of ethanolic and water extracts of *Marchantia linearis* (Bryophyta). The goals are achieved in terms of evaluating total flavonoids, phenolic content and its fractionation by RP-HPLC and HPLC-PAD. Antioxidant potentialities was validated by accessing free-radical (DPPH*, ABTS*, FRAP, phosphomolybdenum, H₂O₂ and ·OH) scavenging, iron chelating activity and scavenging of superoxide radicals were examined. RP-HPLC analysis revealed phenolic acids such as coumarate, sinapate, vanillic and gallic acids. Flavonoids noticed were quercetin, luteolin and apigenin. *M. linearis* extracts presented a remarkable capacity to scavenge all the tested reactive species with IC₅₀ values being found at the µg/mL level. Ethanolic extract was shown to have the highest phenolics while lowest IC₅₀ values for the DPPH*, ABTS* radical scavenging capacities, iron chelating scavenging efficiency, significant activities in scavenging of superoxide radicals, hydrogen peroxide and hydroxyl radicals. The obtained results suggest the potential of *M. linearis* as a potent candidate against free-radical-associated oxidative damage.

Lubaina, *et al.* [9] studied the phytochemical constituents and antioxidant potentialities of petroleum ether, ethyl acetate and water extracts of *Pallavicinia lyellii* a thalloid liverwort. Qualitative aqueous extract analysis of phytochemicals showed the presence of glycosides, tannins, coumarins, alkaloids, saponins, flavonoids, phenols, steroids, reducing sugar and terpenoids. Total flavonoids and phenols were revealed significant levels. Total phenols was fractionated by reverse phase high performance liquid chromatography exhibited phenolic acids such as gallate, chlorogenate, cinnamate, protocatechol, hydroxybenzoate, coumarate, caffeate and ferulate. The *in vitro* antioxidant activity determined through 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant potentiality, hydroxyl radical scavenging activity, superoxide anion radicals quenching effect and metal chelating assay. Among them aqueous extract showed the remarkable free radical quenching activity in terms of FRAP (478 ± 1.76 µM/g), DPPH (89 ± 0.23%) and hydroxyl radical scavenging activity (83.1 ± 0.23%) whereas ethyl acetate fraction exhibited the highest superoxide anion scavenging and iron reduction ability with inhibition rate of 90 ± 0.23% and 25.3 ± 0.96 µg/ml respectively at 1000 µg/ml. The significant antioxidant potentialities may be contributed by the phenolic constituents. The results suggest that *P. lyellii* has promising antioxidant activity and could serve as potential source of natural antioxidants.

Greeshma, *et al.* [10] field surveyed the ignored medicinal bryophytes of the biological world at Neyyar wildlife sanctuary, Trivandrum, Kerala and subsequently scientifically validated the AOX potentialities of *Thuidium tamariscellum*. The analysis includes the phytochemicals screening and its antioxidant potentialities in terms of DPPH, ABTS, H₂O₂, FRAP and metal chelating ability. Total terpenoids level was remarkable. Interestingly, a concentration dependent free radical scavenging potential was noticed and was comparable with the synthetic antioxidant ascorbate. Further, the FTIR analysis of petroleum ether, chloroform, ethyl acetate, and methanol extract confirmed the presence of alcohols/phenols, primary, secondary amines, amides, alkanes, aldehydes, saturated aliphatic alkenes aromatics and aromatic amines, nitro compounds, carboxylic acids, esters, ethers, aliphatic amines, alkyl halides and carbonyls in the moss indicating the presence of medicinally important compounds like flavonoids, terpenoids and alkaloids in the various solvent extracts of the moss. Thus, the overall result of the present study showed that the moss is rich in important pharmaceutical compounds and was reflected as its antioxidant potential. Further studies are warranted to isolate, identify and purify the lead terpenoid present in the moss [11].

Ayyanar and Ignacimuthu [12] documented ethno botanical medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats. Based on the document, Meenu Krishnan, *et al.* [13] analyzed antihaemolytic, anti-lipid peroxidative potential by purified protease inhibitors from the fruits of *Solanum aculeatissimum*. In human erythrocytes against hydrogen peroxide toxicity. Protease inhibitor was isolated and purified from the fruits of *S. aculeatissimum* (SAPI) via four sequential step procedures i.e., salt precipitation to sepharose affinity chromatography. The purity was confirmed by reverse phase HPLC chromatography. The molecular mass was detected using size elution chromatography (22.2 kDa). It has deep roots in history, being one of the major botanicals used in traditional medicine to treat conditions ranging from diabetes, malaria, to snakebites. Subsequently, antihaemolytic and anti-lipid peroxidative potential was carried. Prior to the addition of H₂O₂ to induce haemolysis, different concentrations (50 - 500 µg/ml) of SAPI was added to 2 ml of 4% erythrocyte suspension and allowed to incubate for 5 minutes at room temperature. The mixture was centrifuged and the colour density

of the supernatant was measured spectro photometrically. Quercetin was used as internal standard. The % haemolysis and IC_{50} values were calculated. The PI was potent against haemolysis of the erythrocyte in concentration dependent manner with $IC_{50} = 368.54 \mu\text{g/ml}$. IC_{50} value of quercetin was $224 \mu\text{g/ml}$. The lower the IC_{50} the more protection offered against haemolysis by SAPI. These results suggest that the SAPI is ideal anti-haemolytic agent and offered significant biological action compared with standard drug employed.

Murugan, *et al.* [14] investigated the *in vivo* genotoxicity of *Artemisia japonica* and *A. nilagirica* and its potential antigenotoxicity against cyclophosphamide (CP) induced DNA damage. Initially, the essential oil (EO) was analyzed by GC-MS. *A. japonica* and *A. nilagirica* (250 and 500 mg/kg b.w.) was administered to Swiss white mice of both sexes for 15 days, and the animals received an injection of saline or CP (0.9% NaCl) 24 h before they were euthanized. The GC-MS analysis revealed the presence of camphor, 8-cineole, borneol, artemisia ketone and β -thujone the major compounds in *A. nilagirica*. Meanwhile *A. japonica* contains linalool, caryophyllene oxide, *trans*-linalool oxide, p-cymene and 1, 8-cineole. Significant inhibitions were observed in the evaluated parameters, demonstrating the absence of cytotoxic and genotoxic effects of EOs at all tested doses. In liver, kidney, cardiac and bone marrow cells, EO reduced the DNA damage induced by CP. Results showed a dose-response action of EO. Furthermore, the various tissues showed a difference in the potential antigenotoxic effects. In conclusion, EO of *Artemisia* was not genotoxic and inhibited the genotoxicity induced by CP. Further investigations are needed to purify the potential compound from EO and also to analyze the effects over human health.

Jayakumar, *et al.* [15] evaluated the mitigating effect of purified solasodine from *Solanum mauritianum* against hydrogen peroxide induced oxidative damage in human erythrocytes. *Solanum mauritianum* (Solanaceae) is an exotic species from South America. *Solanum* species are known for alkaloid content which marks them medicinally important. Herbal products have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidant and antitumor potential. Solasodine, the nitrogen analogue of diosgenin, has been reported as potential steroidal precursor for the supplementary source of the commercial synthesis of diverse steroidal drugs. Free radicals are formed during the course of normal metabolic process in the biological system. They are highly reactive molecules due to the presence of unpaired electron. In this juncture, present study was aimed to isolate and purify the alkaloid solasodine from fruits and leaf and to evaluate its antioxidant activity via the assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide, hydrogen peroxide, superoxide anion scavenging activities etc. The alkaloid solasodine was isolated from leaves and fruits of the species using non polar to polar solvents and subsequently purified using column chromatography. Remarkable antioxidant activities are shown by the alkaloid solasodine. IC_{50} values were comparable with synthetic antioxidants such as ascorbate, rutin. Moreover, the solasodine exhibited concentration dependent inhibitory activity against the free radicals. Significant scavenging property was noticed especially with DPPH radicals. Antihemolytic activity of the solasodine was also confirmed from this study. Thus, it can be suggested that solasodine show more protective effects through antioxidant potential against H_2O_2 induced oxidative damage on erythrocytes. So, solasodine can effectively be used as natural antioxidant for the treatment and prevention of lipid peroxidation related disorders.

Similarly, Jayakumar, *et al.* [16] proved the antioxidant and antihemolytic activities of purified caulophyllumine-A from *Solanum mauritianum*. Ripened fruits of *Solanum mauritianum* are used by the local people as vegetable during famine periods and also the fruits and leaves are used to cure various ailments. In this scenario, the present investigation was undertaken to isolate the lead alkaloid molecule from *S. mauritianum* and to evaluate its antioxidant potentialities. In the first phase, the crude alkaloid was isolated, purified by column chromatography yielded a bluish coloured fraction caulophyllumine- A and was further confirmed by NMR. Further, the antioxidant activity was assayed using the DPPH radical scavenging and other assays. The IC_{50} values ranged from 66.5 to $121 \mu\text{g/ml}$. Protective effects of caulophyllumine-A, against H_2O_2 induced oxidative damage in plasmid pBR322 DNA was remarkable at the tested doses ($\mu\text{g/ml}$). Finally, the antihemolytic potential of caulophyllumine- A was analyzed against human blood erythrocytes, whereby the % lysis of RBCs was found to be in the minimal range of 4.5 to 12.4% comparable with the control ascorbate.

Lubaina and Murugan [17] documented the reactive oxygen species and ascorbate-glutathione interplay in signaling and stress responses in *Sesamum orientale* against *Alternaria sesami*. *Sesamum orientale* the wild and cultivar Thilarani exposed to *Alternaria sesami*

infection triggered the signal cascade H_2O_2 content that was positively correlated with lipid peroxidation. The data were also supported by H_2O_2 localization as observed by scanning electron microscopy. Parallely, infection altered chloroplasts marginally and mitochondria effectively in susceptible cultivar than wild sesame. Deformities in the structure of these organelles were accompanied by changes in antioxidant machinery. H_2O_2 can be effectively detoxified via the ascorbate–glutathione cycle. Increases in ascorbate peroxidase, and glutathione reductase activities concomitant with ascorbate (AsA) and glutathione interplay, as well as AsA regeneration ability, function to keep the balance of cellular H_2O_2 under pathogenicity. Dehydroascorbate reductase and monodehydroascorbate reductase are responsible for AsA regeneration. Oxidative damage in Thilarani cultivar compared to wild sesame is attributed by a lower induction of the ascorbate–glutathione cycle as an antioxidant defense system and was not sufficient to protect mitochondria but prevent ultra-structural damage of chloroplasts. Overall, the availability of antioxidants and the induction of antioxidant enzyme activities for detoxifying reactive oxygen species (ROS) are regulated effectively in wild sesame against *A. sesami* induced oxidative stress. The experiments using ROS scavengers demonstrate that the antioxidant defense system is modulated by $O_2^{\cdot-}$ or H_2O_2 signals.

Documenting and validating traditional and indigenous system of medicine in India has been continuously increasing from the last few decades. The current study was made to evaluate the phytochemical constituents and antioxidant potentiality of *Premna serratifolia* an aromatic medicinal plant known as 'Agnimantha' in Ayurvedic system of medicine. It has immense ethno pharmacological importance in folk medicine for curing various human ailments. The qualitative phytochemical screening of the aqueous and ethanolic leaf extracts of *P. serratifolia* revealed the presence of glycosides, flavonoids, alkaloids, tannins, terpenoids and steroids. Subsequently, the quantification of major secondary metabolites revealed remarkable level of phenols followed by terpenoids and flavonoids. The *in vitro* antioxidant activity was determined by DPPH, FRAP, hydroxyl radical scavenging activity, hydrogen peroxide scavenging, superoxide anion radicals quenching effect and metal chelating assay. The aqueous extracts showed positive correlation between concentrations of the extracts with DPPH radical scavenging capacity. Similarly, the other antioxidant potentials also displayed remarkable activities when comparable with synthetic antioxidants such as ascorbate and BHA. The results suggest that the aqueous extract of *P. serratifolia* are potential source for natural antioxidants due to the presence of polyphenols and that could have a protective role as well as prevention from life style diseases [18].

Oxidative stress plays significant role in pathophysiologic events of acute and chronic diseases. Intracellular biomolecules such as lipids, proteins and nucleic acids are damaged via oxidation by excessive ROS. Protease inhibitor was isolated and purified from the fruits of *Solanum aculeatissimum* (SAPI) via four sequential step procedure i.e. salt precipitation to Sepharose affinity chromatography. Subsequently, the antioxidant power is analysed using DPPH, H_2O_2 , $O_2^{\cdot-}$, ABTS, OH^{\cdot} radicals scavenging activity, reducing power potential, metal chelating ability and FRAP (Ferric reducing antioxidant power) method. SAPI exhibited significant IC_{50} values for most of the AOX assays. DPPH radical scavenging, reducing power, metal chelating ability, ABTS and OH^{\cdot} radical scavenging activities were comparable with the synthetic antioxidants like ascorbate and BHT. Further studies are warranted to trace the molecular mechanism of AOX activity by SAPI using *in vivo* animal models [19].

The use of traditional medicine is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of innovative drugs. It is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat various diseases. The systematically performed *in vitro* assays revealed that the tested plant extract may find in therapy as agent with high pharmaceutical value. In this juncture, the present study was undertaken to analyze the antioxidant, antimicrobial and anticancer potentiality of the medicinal herb *Orthosiphon aristatus* Benth. The results reveal marginal antibacterial, anti-inflammatory and anticancer activity than the respective standards. Considering anticancer activity, the extract showed high IC_{50} values for all the cell lines tested. The data on anti-inflammatory activity indicate an inhibition percentage of 19.38, when treated with 10 μ g/ml of extract while indomethacin produced 54.4% inhibition. For antibacterial activity, inhibitory values at higher concentrations were quite good. In general, more activity was obtained when using more concentrated decoctions of the plant extract studied. The best results (60.5% inhibition) were obtained with antioxidant activity. This study provides evidence that the plant extract have an-

tioxidant properties, as tested through the DPPH method. Therefore, the plant may have great relevance in the prevention and therapies of diseases in which oxidant or free radicals are implicated. The ability of the extracts to scavenge the free radical is an indication of the broad spectrum antioxidant potential of *O. aristatus*, which make the plant a candidate for bio prospecting for antioxidant drugs. In addition, the plant is a good candidate for further phytochemical and chromatographic studies to isolate and fully characterize the compound related to this *in vitro* biological activity [20].

Sunila and Murugan [21] aimed to study the changes in the level of phenols and flavonoids during fruit development of *Pouteria campechiana* and its antioxidant potential. The number of experiments conducted was twenty for each case. Polyphenols at different ontological stages may help producers and food technologists to identify which cultivar and/or maturity stage are most adequate for their need. Egg fruits were harvested and classified into six developmental stages based on week after pollination (WAP): stage I (4WAP); stage II (8 WAP); stage III (12WAP); stage IV (16WAP); stage V (20 WAP) and stage VI (24 WAP). The total phenolics and flavonoids of egg fruits at different developmental stages were investigated. The antioxidant capacities of ethanolic and aqueous extracts were determined by different assays such as FRAP, DPPH, ABTS, superoxide anion, hydroxyl radical and H₂O₂ scavenging assays. The total phenolic contents varied from 30.35 to 2.26 mg chlorogenic acid equivalents/g dry weight (DW), and the total flavonoid contents ranged from 0.683 to 3.37 mg rutin equivalents/g DW. Total phenolics showed an initial increase and subsequently decreased during development. In contrast, flavonoids increased from stage I to VI. Antioxidant assays showed varied patterns of inhibition. Significant correlations were observed between antioxidant capacities and total phenolic and flavonoid contents.

Essential oils (Eos) are complex mixtures of volatile lipophilic components and are obtained from leaf, twigs, fruits, flowers of higher plants. These oils are isolated commonly through hydrodistillation. *Pogostemon* species are well known for their essential oils and therapeutic values. Chemical composition and antioxidant activity of Eos from two species viz. *Pogostemon benghalensis* and *P. cablin* were evaluated. Essential oil from both species was extracted by hydrodistillation using Clevenger type apparatus. The essential oil obtained was subjected to GC-Fid analysis followed by GC-MS. The antioxidant activity was studied using DPPH, ABTS, FRAP, metal chelating, hydrogen peroxide, super oxide radical, reducing power, hydroxyl radical scavenging assays using ascorbic acid and butylated hydroxytoluene as standards. 36 to 41 compounds were identified from the essential oil of *Pogostemon* species. α -Cadinol (35.78%) and patchouli alcohol (34.85%) were the major components in these oils. In addition to these compounds 1,8 cineole, aromadendrene, β -patchoulene, α -caryophyllene, β -caryophyllene, α -patchoulene, germacrene A were identified as the other predominant compounds. The antioxidant activity of Eos was significant when compared with standards. 75.3 to 91.5% inhibition activity was noticed against various free radical scavenging assays. The Eos showed remarkable IC₅₀ value which suggests their potency to use as a natural antioxidant in various industries, foods and therapeutics [22].

Ethnic usage reveals that most of the plants possess a wide array of biological and pharmacological potentialities that may protect tissues from oxidative damages. In the present part, various solvent extracts of root and leaves of *Thottea siliquosa* was screened to evaluate their potent *in vitro* antioxidant activity, total phenol, alkaloid, saponins and flavonoid contents in order to find possible sources for future novel antioxidants for pharmaceutical formulations. Thorough study was performed with the petroleum ether and ethyl acetate extracts of root and leaf of the plant by *in vitro* chemical analysis and antioxidant potentialities. Preliminary qualitative analysis revealed the presence of alkaloids, tannins, glycosides, coumarins, flavonoids and saponins. Phenols (2.5 - 4.8 mg/g), alkaloids (43 - 287 μ g/g) and saponins (66-303 μ g/g) represented the highest level both in the petroleum ether and ethyl acetate extracts of root and leaves. The antioxidant potentialities in terms of DPPH radical, hydroxyl radical, superoxide anion and nitric oxide quenching capacity revealed varying responses. The IC₅₀ values are comparable with the standards such as ascorbate and butylhydroxy toluene. Lipid peroxidation level was comparatively inhibited by the extracts. Results from present study suggest that the phytochemicals in the extracts of *T. siliquosa* act as antioxidant agents as it was substantiated by its free radical scavenging activities [23].

Artemisia species are used in traditional medicine for the treatment of various ailments such as indigestion, infection, irregular menstruation, cramp, cold, epilepsy, typhoid, tuberculosis, urinary calculi, colic, fever, asthma, bronchitis, sciatica, flatulence, anaemia, insom-

nia, gout, depression, nervousness, hysteria, measles, bruise, chilblain, leprosy, malaria and cancer. Similarly, most species has been used as antiseptic, antioxidant, anthelmintic, expectorant, diuretic and insect repellent. The biological activity shown by the plants was due to the essential oil present in them. The present study describes the fractionation of essential oil and its antioxidant potentialities in two allied species - *Artemisia japonica* Thunb. and *A. nilagirica* (Clarke) Pamp. The essential oil from *Artemisia japonica* and *A. nilagirica* was isolated by hydrodistillation and analyzed by GC-MS. 30 and 19 compounds were noticed in the oil respectively. The predominant phytochemicals in the essential oil of *A. nilagirica* were camphor (41%), 8-Cineole (17.0%), Borneol (8.6%) Artemisia ketone (6.4%) and β -Thujone (6.0%). Meanwhile *A. japonica* contain Linalool (70.4%), Caryophyllene oxide (6.7%), *trans*-Linalool oxide (4.5%), p-cymene (3.4%) and 1, 8-Cineole (2.3%). Significant antioxidant potentialities was displayed in terms of DPPH scavenging, beta carotene bleaching, total antioxidant activity, soybean oil rancidity and sun flower oil free fatty acids and iodine values. Interestingly, *A. nilagirica* showed remarkable antioxidant potentialities and was comparable with synthetic antioxidants such as ascorbate, butylated hydroxytoluene and BHA. The present study suggests that the tested *Artemisia* species revealed a pool of potential biomolecules in the essential oil and also exerted remarkable antioxidant values. Further studies are warranted to isolate and purify the principle component from the essential oil [24].

Tylophora subramanii, a climbing endemic species of Southern Western Ghats belongs to Asclepiadaceae. The local people use this plant for curing many rheumatic ailments. The objective of this study is to explore the phytochemistry and the antioxidant potential of ethanolic leaf extract of *T. subramanii* which is considered traditionally as an important medicinal plant. The methods adapted includes (a) phytochemical analysis to find out the presence of various bioactive compounds (b) *in vitro* antioxidant analysis of ethanolic leaf extract by 2,2-diphenyl-1-picrylhydrazyl assay, nitric oxide scavenging assay, superoxide scavenging potential, *ex vivo* superoxide dismutase assay, catalase assay. The major outcomes are the following: ethanolic leaf extract showed remarkable levels of the phytochemicals such as flavonoids, tannins, terpenoids, saponins, alkaloids, proteins and carbohydrates. Similarly, it also showed potential antioxidant activities. High catalase and superoxide dismutase activity with low lipid peroxide level, which were comparable with the synthetic antioxidant L-ascorbic acid. Thus, the work concludes that *T. subramanii* display a wide range of pharmacologically useful phytochemicals which exhibited significant antioxidant potentials. Future works are planned to elucidate the lead molecule and its biological potentialities [25].

Aswathy and Murugan [26] documented the ethnobotanical knowledge about herbals used by tribes of Kerala and Tamil Nadu with special reference to *Begonia malabarica*. Anthocyanins from vegetables and flowers have fascinated pharmaceutical industries are proven nutraceutical health. *Begonia* species forms diverse hyper group and are distinguished on the basis of morphological parameters. *Begonia rex-cultorum* (Baby rainbow) and *Begonia malabarica* exhibited the highest antioxidant activities. The anthocyanin concentration positively correlates with the antioxidant potentialities among the cultivars. The IC_{50} value related with DPPH and metal chelating activities of the *B. rex-cultorum* (Baby rainbow) extract are 32.3 $\mu\text{g}/\text{mL}$ and 18.7 $\mu\text{g}/\text{mL}$ respectively. Remarkable scavenging potentialities are displayed against metal chelating, carotene bleaching, ABTS radical, FRAP assays and the results are comparable with synthetic antioxidant like BHT. The diversity in radical scavenging in these assays may be due to factors like stereo selectivity of the radicals or due to the differential solubility of anthocyanin molecules in the crude extract. Further studies are warranted to isolate and fractionate the major anthocyanins in the cultivars [27].

Bosco Lawrence and Murugan [28] reported the folklore use of *Osbeckia* species from Munnar hills, Kerala and subsequently validated the information. Health-benefit properties of natural pigments have been intensely studied, especially the anthocyanins. In the last few decades, research on anthocyanins has attracted biologists by the increasing evidence of their health beneficial effects. *Osbeckia*, belongs to Melastomataceae and is well-known for colouring pigments and other bioactive compounds. In the present study, total anthocyanin and antioxidant capacity indicators were evaluated from 8 *Osbeckia* spp. and anthocyanin was extracted from *in vitro* cultures of *O. aspera* and *O. reticulata*. The antioxidant effect was studied using ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay, the FRAP, the scavenging ability of hydroxyl radicals and the superoxide anion scavenging activity. Anthocyanins extracted from *in vitro* cultures were purified and fractionated using column chromatography and LC-MS MS analysis. *In vitro* cultures

of *O. aspera* was obtained in MS medium fortified with various combinations of Benzyl Adenine (BA), Naphthalene acetic acid (NAA) and 2, 4-D. The chromatograms of *O. aspera* revealed the presence of malvidin-3 -diglucoside, peonidin, delphinidin and cyanindin whereas *O. reticulata* cultures accumulated large amounts of malvidin, cyanindin and cyanidin aglycone. Thus, the results tempt to suggest that the *Osbeckia* species are rich in anthocyanin and therefore displayed potential AOX power. *O. aspera* and *O. reticulata* callus was induced for the *in vitro* production of anthocyanins. The pool of anthocyanins was purified and fractionated by LC-MS/MS and AOX assays were performed with the purified anthocyanin which showed higher level activities [29].

Greeshma Murukan and Murugan [30] reported the natural dye yielding plants used by the tribes of Wayanad, Kerala, India. They validated the hepatoprotective and antioxidant activity of purified anthocyanin extracted from the cell suspension culture of *Clerodendron infortunatum*. A protocol has been developed for the induction of callus proliferation from leaf and nodal explants of *C. infortunatum*. The explants were inoculated on murashige and skoog (MS) medium supplemented with diverse combinations of 2, 4-dichlorophenoxyacetic acid (2,4-D) and benzylaminopurine (BAP) for triggering callus formation. Subsequently, the green compact callus has been sub-cultured in the medium fortified with 2,4-dichlorophenoxyacetic acid (2,4-D) and Kinetin for anthocyanin synthesis. Cell suspension culture was also established and the elicitor, salicylic acid was used for triggering anthocyanin synthesis. Three different chromatographic columns (solid phase extraction by Sepharose C18 column, Oasis-MCX and Amberlite XAD 7+Sephadex LH 120 sorbents) were employed to purify the *in vitro* synthesized anthocyanin from cell suspension cultures. For purity evaluation, high-performance liquid chromatography (HPLC) and molar absorptivity assay was used. Further, hepatoprotective and antioxidant activity was evaluated comparing with silymarin, as standard in rats. *In vitro* antioxidant scavenging activity was analysed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay. After 1 month, the leaf explants yielded remarkable green compact callus on murashige and skoog (MS) medium containing 2.0 mg/l benzylaminopurine (BAP) and 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). Salicylic acid enhanced anthocyanin synthesis. The mean purity values obtained by high-performance liquid chromatography (HPLC) were $90.9\% \pm 1.9$ and $80.60\% \pm 2.3$ for Oasis MCX, Amberlite XAD-7+Sephadex LH-20 column respectively. However, the purity calculated by molar absorptivity was found to be less. The highest purity achieved using molar absorptivity analysis was with MCX cartridges i.e., $85.9 \pm 3.8\%$. High-performance liquid chromatography (HPLC) yielded 12 anthocyanin fractions. Remarkable antioxidant scavenging activity was noticed as revealed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay. The hepatoprotective activity (25, 50, 100 mg/100g b. w) was compared with silymarin (25 mg/kg b. w) against carbon tetrachloride (CCl_4) induced toxicity. Anthocyanin extract improved the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and recovered the activity of kidney function by decreasing the urea and creatinine content. In addition, the administration of anthocyanin significantly inhibited the oxidative stress via its scavenging of the reactive oxygen species formed by carbon tetrachloride (CCl_4) stress. Further, a decrease in the malondialdehyde (MDA), hydrogen peroxide (H_2O_2), nitric oxide (NO) accumulation and an increase of glutathione (GSH) content were noticed. Similarly, improved lipid profiles, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were also observed suggesting that anthocyanin significantly suppress the toxicity via its activation of antioxidant enzymes [glutathione transferase (GST), catalase (CAT) and superoxide dismutase (SOD)]. The overall results showed that the purified anthocyanin of *C. infortunatum* functions as an antioxidant and thereby hepatoprotective protection against carbon tetrachloride (CCl_4) induced toxicity in animal models [31].

Greeshma Murukan and Murugan [32] purified and characterized anthocyanin from *in vitro* culture of teak and its antioxidant potential. Anthocyanin was extracted from *in vitro* culture, purified by using amberlite lite XAD column and fractionated by Liquid chromatography mass spectrometry (LC-MS/MS). Various antioxidant assays were carried such as 2,2-diphenyl 1-picryl-hydrazyl-hydrate (DPPH), 2,2'-azino-bis-3-ethyl-benzothiazoline-6-sulphonic acid (ABTS), Oxygen radical absorbance capacity (ORAC), Nitric oxide (NO) and Hydrogen peroxide (H_2O_2). Liquid chromatography mass spectrometry (LC-MS/MS) revealed the major fraction as cyanidin 3-(2-xylosyl-rutinoside) with unknown peaks. The amount of anthocyanin was 15.23 mg/g monomeric anthocyanin. Further, the potential antioxidant capacity of the teak anthocyanin was comparable to common vegetables and fruits. Similarly, high correlations of anthocyanin with

antioxidant activity, such as oxygen radical absorbance capacity (ORAC), 2,2'-azino-bis-3-ethyl-benzothiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) ($r = 0.95, 0.93, \text{ and } 0.80$) were found. The high anthocyanins content and potential antioxidant activity suggests that teak anthocyanin may be applied in the food industry as a good source of natural pigments.

Arathy, *et al.* [33] evaluated the antioxidant activities of purified anthocyanin from wild balsam species. Initially, anthocyanin was extracted from floral leaves of wild balsam species and purified by chromatographic techniques. The major fractions identified were hesperidin, dimethoxy antirrhinin and trimethoxy antirrhinin. Further, the anthocyanin extracts were subjected to *in vitro* protocols like 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation, DPPH scavenging assay, ferric reducing antioxidant power (FRAP), beta carotene bleaching assay, metal chelating and H_2O_2 scavenging power. Interestingly, ABTS, FRAP analyzes yielded significant results as compared to others. The data were comparable with that of synthetic antioxidants like ascorbate and catechin. Meanwhile, beta carotene and H_2O_2 scavenging assay showed moderate results. DPPH and metal chelating protocols displayed the values 71% and 64% respectively at 25 $\mu\text{g}/\text{ml}$ concentration. The antioxidant results were supported by NMR and LC MS analysis. This study provides model systems for the evaluation of natural antioxidants like anthocyanin. Future *in vivo* clinical studies are warranted to confirm the obtained data.

Conclusion

The overview of the present report infers that these plant extracts possess optimal antioxidant properties. Thus, the extracts can be beneficial in treating stress caused due to multiple of factors. Over the past few years, significant scientific knowledge has been documented regarding plant redox characters and its AOX defense. Plants synthesize and accumulate many non-enzymatic antioxidants like ascorbate, glutathione and polyphenolics. Some of these AOXs occur constitutively, while others are synthesized in response to abiotic and biotic stress events. Almost all plant based phytochemicals exhibit certain level of antioxidant activity under *in vitro* assays. However, for *in vivo* studies, plant antioxidants have to channel through multiple process. However, many pharmaceutical industries tapping such native knowledge of people in to economically important nutraceutical products. In this juncture, the above said species could be further screened for isolating unique antioxidants that can be supplemented to human diet.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgements

The authors thank the study group for their collaboration.

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Volume 9 Issue 1 January 2021

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