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A COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY BY TWO DIFFERENT SPECIES OF ALGAE

Prathima Mathias D. A.*[1], Halesh S.[2], Latha R. [2], Ram Prasad B. R.[2], Smitha C. R.[2], Taramani K.[2] and Vidyashree Y. E.[2]

[1]Associate Professor, Department of Chemistry, I.D.S.G Govt. College, Chikkamagalur – 577102, Karnataka, India.
[2]Department of Chemistry, I.D.S.G Govt. College, Chikkamagalur – 577102, Karnataka, India.

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***Corresponding Author**
Prathima Mathias D. A.
Associate Professor,
Department of Chemistry,
I.D.S.G Govt. College,
Chikkamagalur – 577102,
Karnataka, India.

ABSTRACT

The present study has given a novel method for the effective use of non-endangered (i.e., abundant) plants and plant parts as antioxidants. Vesicularia dubyana, Spirogyra majuscule (Pond) and Spirogyra majuscule (trees) were chosen for this study. Both Sohxlet and sonication methods of extraction were adopted. The extracts were subjected to biochemical analysis. Protein, phenol, reducing sugar, Flavanoid, antioxidant assay and reducing power assay were determined spectroscopically. Moisture, ash, protein, ether and reducing sugar percentages were evaluated by routine methods.

KEYWORDS: *Vesicularia dubyana*, *Spirogyra majuscule*, biochemical analysis, antioxidant activity and reducing power assay.

1.0 INTRODUCTION AND LITERATURE REVIEW

A majority of 94% energy used within the transportation sector originates from petroleum. Heavy dependence on petroleum based fuels, is not sustainable due to increasing fuel costs, diminishing crude oil reserves and the environmental impact of fossil fuel usage (Chisti, 2007; Pienkos and Darzins, 2009; Demirbas and Fatih Demirbas, 2011). Energy demand worldwide continues to increase at a rapid pace with developing countries, such as China and India's, energy consumption rates rising at 2 to 3 times the global average (Ahmad et al., 2011). Crude oil reserves are being depleted (Abdullah et al., 2007) at a rate of approximately 85-90 million barrels of oil per day. With worldwide proven oil reserves estimated at 1.3 trillion barrels of oil, it is possible that crude oil will be depleted within the next 50 years (Abdullah et al., 2007).

Nowadays, the petrochemical resource shortage and environmental pollution are two critical challenges, which need to be addressed by our society. In order to solve these problems, both social and industrial researchers have started looking for renewable energy alternatives that can partially replace fossil fuel resources for establishing a more sustainable society and promoting economic recovery in the world.

Biomass energy, as one of the representatives of renewable energy, has been developing rapidly in form of agriculture and aquaculture. Biomass energy is usually produced by terrestrial crops and marine algae for conversion into biofuel and biogases. Since the 1970s, the United States and Brazil have begun to develop biofuel by using corn to produce bio-ethanol, and achieved significant results (Sims and Taylor, 2008). Biomass energy cannot only reduce the energy shortage problem, but is also much better for the environment than fossil fuel. However, terrestrial crops - the first and second generation biofuel feedstocks pose new challenges such as the occupation of arable land which end in food crisis. Thus one of the most serious concerns regarding sustainability aspects today is the need of land to produce food to an increasing population. There is average of 25,000 people that die of hunger every day in the world, according to a report to FAO. In other words, one person dies of hunger every 3.4 seconds. (Burton, 2011).

Biomass can be extracted from plants, animals and microorganisms such as food crops, crop wastes, wood, garbage, animal manure, marine algae, etc. The main advantages of Biomass are renewability and less pollution. Biofuel and biogases are produced from biomass as an alternative petroleum fuels. At present development of biomass has become an important way to adjust energy structure and to reduce greenhouse gas emissions in order to realize both environmental and economic sustainability (Wu et al, 2007).

Recent focus of scientist worldwide is on the microalgae biofuel development. A general lack of commercial value, small size, and inconspicuous place in the ecosystem have made the bryophytes appear to be of no use to most people. However, Stone Age people living in what is now Germany once collected the moss *Neckera crispa*. Microalgae have less complex structure, fast growth rate and high oil content characteristics. As Algae production need less land or can even be cultivated in the shallow sea directly, it can help us saving arable land to grow food. Therefore, microalgae are more sustainable than terrestrial crops. Algae will probably play an increasing role in the sustainable energy use field in the near future (Patil and Tran, 2008).

Antioxidant activity of algae

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's).

The recognized dietary antioxidants are vitamin C, vitamin E, selenium and carotenoids. However recent studies have demonstrated that flavonoids found in fruits and vegetables may also act as antioxidants. The capacity of flavonoids to act as antioxidants (free radical scavenging activity) depends upon their molecular structure and the position of hydroxyl groups (Ameur Elaissi etal, 2012).

Structurally diverse mixtures of small molecules function well in phenotypic assays. Marine algae represent a rich source of structurally diverse small molecules including essential vitamins and minerals, polyunsaturated fatty acids and antioxidants (Pomponi S.A: 1998, Montaser R, Luesch H. 2011). Red algae continue to be a source of natural product discovery with 42 new molecular structures identified in 2011.

In view of the above literature survey, we found that the algae *Spyrogyra majuscule* (grown in plants and on trees) and *Vesicularia dubyana* (Java moss) found in the fresh water ponds of Chikmagalur can be investigated as good sources of biodiesel and antioxidants along with their bio-compositional studies.

Spyrogyra species are reported to exhibit antioxidant activites (Koksal Pabucu *2012*, Mayalen Zubia 2007), and few species have been reported to produce biofuel (Ramachandra T.V. etal *2013).*

2.0 MATERIALS AND METHOD

2.1 SAMPLE COLLECTION AND IDENTIFICATION

Algal samples were collected from ponds of Sakarayapatna of Chickmagalur District, Karnataka at depths of 2-20 m. The date and location of each collection was recorded along with a photograph, and a formalin preserved sample. Specimens were identified by comparing morphological traits to that of known algae.

2.2 GENERATION OF ALGAL EXTRACTS

Freshly collected algae (each sample measuring 20 mL by volumetric displacement) were extracted with successive exposure to methanol for 6-16 hours. Crude extracts were filtered to remove insoluble material and the methanol removed *in vacuo*.

2.3 BIOCHEMICAL ANALYSIS

The following estimations were carried out by standard methods (H.A. Abd El-aal and F.T. Halaweish 2013), details are enlisted in Table-1. All experiments were carried out in triplicate and averaged.

Table -1: Table of estimations.

Sl. No.	PARAMETERS ESTIMATED	Method
1	MOISTURE	Dry weight method
2	TOTAL ASH	Ignition method
3	ETHER EXTRACT	a) Sohxlet method (T-1) b) Sonication method (T-2)
4	PROTEIN	Lowry's method
5	TOTAL PHENOL	Spectrophotometric method
6	REDUSING SUGAR	DNS method
7	FLAVANOID ASSAY	Reducing method
8	ANTIOXIDANT ASSAY BY DPPH METHOD	DPPH method

3.0 RESULTS AND DISCUSSION

Table-2 summarizes all biochemical analysis reports obtained by following standard protocols mentioned in Table-1.

4.1 ESTIMATION OF MOISTURE

Moisture content has significant effect on the engineering of the conversion process: either thermochemical (combustion, gasification or pyrolysis), or biochemical (fermentation and anaerobic processes). Increase in moisture content of biomass from 0% to 40% decreases the heating value by about 66%. Biochemical conversion (eg anaerobic digestion) are able to process effectively only at high moisture content (Aguilera-alvarado, 2013, and Niaz M. and Rasul E.,1998).

Spirogyra majuscule of pond showed highest moisture content since it is an aquatic algae. Whereas the other algae have also shown considerable water content in them. Water in algae varies from region to region and seasonally. The observed result may be due to the moist climatic conditions in Chickmagalur. The algae were collected in the beginning of summer season, yet there was considerable amount of moisture content in them. *Spirogyra majuscule* therefore has biochemical importance and can be a very good material for vermicompost, whereas the other algae may be effectively utilized in thermochemistry with slight modifications.

Table -2: Bio chemical composition (%), total phenol, flavanoid and antioxidant content.

Part	Moisture %	Ash %	Type of extract	Pet ether %	Protein %	Reducing sugar %	Total phenol	Flavanoid	Antioxidant
							(mg GAE/100g DW)		
V.dubyana	5.95	17	T-1	75	1.1	25.9	900	1300	6.8
			T-2	72.72	3.4	20.4	100	2600	40.0
S. majuscule (POND)	26.65	7.0	T-1	12.9	4.3	27.1	1000	200	46.8
			T-2	28.5	1.4	26.8	100	500	16.4
S. majuscule (TREE)	4.96	14.0	T-1	14.0	3.7	16.5	950	100	47.4
			T-2	5.63	1.1	20.5	1050	900	14.2

4.2 ESTIMATION OF ASH

The quality and quantity of ash in biomass depends on a large amount of factors including its derivation, growing and harvest conditions, the fertilization type, the harvest techniques, its storage and transportation along with its pretreatment before it is introduced into bioenergy conversion process. Lower ash content values are desirable for thermochemical processes (combustion, gasification or pyrolysis), however the ash content effect on biochemical processes (fermentation and anaerobic processes) is still not defined (Aguilera-alvarado, 2013, and Niaz M. and Rasul E.,1998).

The average energy value of ash free, oven-dried plant residues is about 4.7kW/kg, at 15% moisture content, the energy value of the ash free residue is about 4.2 kWh/kg. Ash content at a level of 2% will reduce the energy value to about 3.9 kWh/kg.

Spirogyra majuscule (pond) has very low ash content and therefore can be good in thermochemical processes. *Vesicularia dubyana* and *Spirogyra majuscule* (trees) have considerable ash content and they can be used in biochemical processes. Scope of using these algae as biosorbants is yet another interesting and wide application oriented field.

4.3 ESTIMATION OF PETROLEUM ETHER EXTRACT

Petroleum ether dissolves fats, muscilages and chloroplasts. These substances affect phytochemical activity to a large extent. However if the petroleum ether extract is more, then it would become a good source of raw material for soap manufacturing industry (Sun Y, Qiao L, 2013).

Vesicularia dubyana showed considerable fats extracted with petroleum ether and can be used in biodiesel production by transesterification. *Spirogyra majuscule* showed less ether extract in comparision to *V. dubyana.*

4.4 ESTIMATION OF PROTEIN

Protein is most vital biochemical molecule which forms the body mass of living beings. These proteins exist in dissociated forms like amino acid, peptide, polypeptide, enzyme or hormones. Protein is indicator of biological activity, hence in any biological reaction or estimation or bio-process, protein analysis and quantification is done to determine the quantity, quality of protein and thereby the state of biological reactions (Anne Pihlanto 2006, Gulizar Atmaca 2004). Hydrolysis method gave total protein analysis report.

4.5 ESTIMATION OF TOTAL PHENOL

Natural phenols chemically interact with many other substances, stacking a chemical property of molecules with aromaticity. These are reactive towards oxidation and undergo auto oxidation during ageing process. The naturally occurring phenols reduce antioxidant properties to considerable extent, hence it is highly important to investigate the phenolic content of the active formulation (Shakthi Deve A, 2014, Seria Zootehnie. 2013). In our present investigation, *Spirogyra* of trees has highest phenolic content, in both hot and sonicated processes, 950 mg and in the green method the same was found to be 1050mg. The high phenolic content may hamper antioxidant activities of *Spirogyra* whereas the java moss may show considerable activity.

4.6 ESTIMATION OF REDUSING SUGAR

Reducing sugars have carbonyl groups and acts as reducing agents. Presence of reducing sugars in extracts makes them powerful antioxidants (Carbohydrate assay). In FS method, the observed quantity of reducing sugars was higher, when compared to DNS method. *S. majuscule* (Pond) gave higher reducing sugar in FS method. The values of reducing sugars obtained are as given in table-11 and figure-10.

Java moss showed 25.9 and 20.4 percent of reducing sugars in hot and sonicated extracts. The FS method gave more reliable results in comparison to DNS method (Because, the total biomass will be nearly equal to 100%).

4.7 ESTIMATION OF FLAVONOID

The flavanoids are shown to be antioxidative (Skerget M 2010, Wang M, Tsao R, 2006). From the Table-13, the sonication method has given higher Flavanoid content in case of *V. dubyana* and *S. majuscule* (pond and tree samples). Hence we can expect the enhanced in the antioxidant activity of these algae. Further, these results showing considerable antioxidant activity of these algae due to Flavanoid content, is as shown in the Table-14.(Nagao A. 1992, Gupta P 2012, Bashir S 2008, Gong Y 2012 2011, Durand Denys PATENT:EP1902631 and Ying Gong Xuan Liu 2012).

4.8 ANTI OXIDANT ASSAY

The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages. The Table-2 shows the antioxidant activity of the selected algae. Polyphenols (flavonoids) and other phenolic contents estimated from the sonication method show hamper in antioxidant property. *V. dubyana* hot extract, *S. majuscule*. pond and tree sonicated extracts show less antioxidant activity due to high phenolic content in them.

The highest concentration of reducing sugars and proteins posses greater reducing powers (carbohydrate assay), the result revealed in the present study that hot extracts of *V. dubyana* and *S. majuscule* (pond) have highest reducing sugar concentration as shown by FS method of analysis. Hence, the above results support the fact that higher concentration of reducing sugars, in turn increase antioxidant activity of the tested extracts as reported in Table – 2. The protein content obtained from hydrolysis method shown in Table – 2 may also support the antioxidant results.

5.0 CONCLUSION

The pond sample of *S. majuscule* has good reducing and antioxidant property in comparison to the tree sample of same species and *V. dubyana.*

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