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Phytochemical Screening and Antioxidant Potential in the Leaf Extract of Annona Muricata

Nalini. P[1], Brindha Durairaj[2]
[1,2]*Department of Biochemistry, PSG College of Arts & Science, Coimbatore, India.*

Abstract: ***The objective of the study was designed to screen the preliminary phytochemicals and to determine the antioxidant potential in the hydroethanolic leaf extract of Annona muricata. The phytochemical analysis revealed the presence of selected phytoconstituents (flavonoids, phenols, tannins, saponins, steroids, glycosides and alkaloids). Activity of Enzymic antioxidants (superoxide dismutase, catalase, peroxidase and glutathione reductase) in the leaf extract was observed to be 24.2, 3.08, 7.40 and 20.37U/g respectively. Nonenzymic antioxidants such as ascorbic acid (22.5 mg/g), α-tocopherol (3.25 mg/g), carotenoids (2.64 mg/g), reduced glutathione (1.64 mg/g), phenols (11.8 mg/g), flavonoids (1.64 mg/g), tannins (2.84 mg/g) and carbohydrate (18.69 mg/g) were found to be rich in the hydroethanolic extract. The results obtained in the present study revealed that Annona muricata leaf extracts proves to be potential source of natural antioxidant and hence justifies its use in folkloric medicine which might be used for further investigation.***

Key words: Phytochemicals, Annona muricata **leaves** **,** ***enzymic and nonenzymic antioxidant, hydroethanolic extract***

I. INTRODUCTION

Phytochemicals are bioactive compounds found in plants which are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. Phytonutrients have various health benefits (Antimicrobial, anti-inflammatory, anticancer, antidiabetic and antihypertensive effects). The phytochemical constituent of a plant will often determine the physiological action on the human body [1].

Antioxidants mean "against oxidation" which can retard any form of oxidative/nitrosative stress or its consequences. A highly complex array of antioxidant system has been developed by humans to cope up with oxidative stress and protect the body against free radical damage. The antioxidants can be endogenous or exogenous in origin. There is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases [2].

Annona muricata L. commonly known as graviola or soursop belongs to the family Annonaceae. Traditionally, the leaves are used in the treatment of headache, insomnia, cystitis, liver problems, diabetes, hypertension and are proved to possess various beneficial effects [3]. The present study was carried out to screen the phytoconstituents and to evaluate the antioxidant potential of medicinally important Annona muricata leaf extract.

II. MATERIALS AND METHODS

A. Collection and extraction of Plant Material

The plant sample (leaves) of *Annona muricata* were collected from Coimbatore. The leaves were authenticated by the Head, Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2015/Tech/153). The leaves were washed with water and shade dried at room temperature. The dried leaves were ground into fine powder using mixer grinder.

B. Preparation of Extract

25g of the leaf powder was soaked in 250 ml of hydroethanol, agitated manually and left undisturbed for 72 hours. It was then filtered using Whatmann No.1 Filter paper and filtrates were evaporated. The extracts were stored at 4°C for further use.

C. Qualitative Analysis of Phytochemical Screening

Different solvent Extracts were used to screen the selected phytochemicals such as phenols, flavonoids, alkaloids, tannins, steroids, saponins and glycosides. Procedures for these studies were performed according to the published standard methods [4].

Test solution was prepared at 10% (w/v) concentration in distilled water unless otherwise mentioned in individual test. This process was carried out by using the standard protocols.

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D. Estimation of Enzymic and Nonenzymic Antioxidants in Annona Muricata

The enzymic antioxidants like superoxide dismutase [5], catalase [6], peroxidase [7] and glutathione reductase [8] were determined in the hydroethanolic leaf extracts of *Annona muricata* using the standard methods. The nonenzymic antioxidants like ascorbic acid, α-tocopherol and carotenoids were evaluated by the method of Sadasivam and Manickam [9]. The total reduced glutathione were estimated by Boyne and Ellman [10] method. The phenol [11], flavonoids [12], tannin [13] and carbohydrate [14] content were determined using standard protocols.

E. Statistical Analysis

Data were expressed in mean ± Standard Deviation. All the assays were performed in triplicates.

III. RESULTS AND DISCUSSION

Phytochemicals are non-nutritive plant chemicals with disease preventive properties in humans [15]. The preliminary phytochemical analysis of different solvent extracts were revealed the presence of phenols, flavonoids, alkaloids, tannins, steroids, saponins and glycosides as depicted in the table 1.

Table 1: Phytochemical screening of hydroethanolic extract of Annona muricata leaves

S no	Phyto Constituents	Petroleum ether extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract	Hydroethanolic Extract
1	Carbohydrate	+	+	+	+	+
2	Protein	+	+	+	+	+
3	Flavonoid	+	+	+	+	+
4	Steroids	-	-	-	-	-
5	Saponins	-	-	+	-	+
6	Phenol	+	+	+	+	+
7	Glycosides	-	-	-	+	+
8	Tannin	-	-	-	+	+
9	Alkaloids	-	-	-	+	+

Abbreviations: (+ Indicates Presence, - Indicates Absence).

The results clearlyindicated that the majority of the phytochemicals were present in all the selected solvent extracts. However, the hydroethanolic leaf extract of Annona muricata was found to contain all the active phytoconstituents when compared with other extracts. These qualitative observations offer a scientific validation for the lead compound identification and for further in vitro and in vivo study. Our results are on par with the work done by Parekh et al., 2006 reported that the methanolic extracts possessed various phytochemicals in Bauhinia Variegate L. bark [16].

A. In vitro Antioxidant Potential of Hydroethanolic leaf Extract of Annona Muricata

Antioxidants are the key regulators to counterbalance the effect of oxidants and reduce the risk for many pathological conditions [17]. The enzymic and nonenzymic antioxidant systems play a decisive role in protecting cellular membranes and organelles from the damage caused by reactive oxygen species [18]. A variety of enzymic and nonenzymic antioxidants were evaluated and presented in the table 2 and 3.

1) *Enzymic Antioxidants:* Superoxide dismutase (SOD) is a vital antioxidant which neutralises oxyradicals by accelerating the dismutation of superoxide to hydrogen peroxide. Catalase (CAT) is another antioxidant which is involved in the removal of hydrogen peroxide formed during the reaction catalyzed by SOD. Thus, SOD and CAT act synergistically and provide protective defense against reactive oxygen species [19]. In the present observation, the CAT activity in hydroethanolic leaf extract of Annona muricata was found to be 3.08 U/g and while the SOD activity was observed as 24.2 U/g respectively.

Peroxidases are heme containing enzymes that are able to oxidize organic and inorganic compounds using hydrogen peroxide as cosubstrate. The non-specificity of peroxidase makes the enzyme suitable to a broad range of electron donor substrates. Glutathione reductase acts by increasing the level of reduced glutathione and plays an important role in protecting hemoglobin and biological

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cell membranes against oxidative damage [20]. Peroxidase and Glutathione reductase activity in hydroethanolic leaf extract of Annona muricata was found to be 7.40 and 20.37 U/g respectively.

Our results are in good correlation with the work done by Starlin and Gopalakrishnan, 2013 who reported that the hydroethanolic extract of Tylophora pauciflora showed significant activities of enzymic antioxidants [21].

Table 2: Quantification of Enzymic Antioxidants of Annona muricata Leaves

Enzymic Antioxidants	U/G*
Superoxide Dismutase	24.2 ± 8.26
Catalase	3.08 ± .09
Peroxidase	7.40 ± 0.13
Glutathione Reductase	20.37 ± 2.8

[Values are expressed as mean ± Standard deviation (n=3) Units: Superoxide dismutase – Units/mg protein: Catalase - µmole of H_2O_2 of consumed/min/ mg protein. Peroxidase -µmoles/g tissue Ascorbic acid-µg/mg protein; α –tocopherol-µg/mg protein; Carotenoids-µg/mg protein.]

2) *Nonenzymic antioxidants:* Ascorbic acid is a water soluble vitamin which can neutralize ROS before lipid peroxidation is initiated [22]. α-Tocopherol is a major lipid soluble antioxidant which is capable of preventing the chain propagation reaction by repairing oxidizing radicals [23]. The ascorbic acid and α-Tocopherol content in hydroethanolic leaf extract of *Annona muricata* was observed as 22.5 and 3.25 mg/g respectively.

Carotenoids and its metabolites are efficient antioxidants in quenching reactive oxygen species and have been associated with a decreased risk of life threatening diseases [24]. Reduced glutathione (GSH) is a major non-protein thiolcapable of preventing cellular damage caused by reactive oxygen species [25]. The carotenoid and GSH level in leaf extract was noticed to be 2.64 and 1.64 mg/g respectively.

Our results are in par with the work done by Gomathi et al., 2012 who reported that the hydroethanolic extract of Evolvulus alsinoids possessed maximum nonenzymic antioxidant content [26].

Flavonoids, phenols and tannins are the most widespread secondary metabolites which received much attention as a potential natural antioxidant as they efficiently scavenge free radicals [27].The phenol, flavonoid, tannin and carbohydrate contents in hydroethanolic leaf extract of Annona muricata were found to be 11.8, 1.64, 2.84 and 18.69 mg/g respectively. Our results are in good accordance with the work done by Vennila and Brindha, 2014, who reported that the Morinda citrifolia fruit extract were found to possess various phytoconstituents [28].

Table 3: Quantification of nonenzymic antioxidants of Annona muricata leaves

Nonenzymic antioxidants	Mg/g*
Ascorbic Acid	22.5±1.0
A –Tocopherol	3.25±0.5
Carotenoids	2.64±0.61
Reduced Glutathione	1.64±0.06
Phenol	11.8 ± 0.13
Flavonoid	1.64±0.02
Tannin	2.84± 0.16
Carbohydrate	18.69 ±0 .12

*Mean±SD

IV. CONCLUSION

From our study, it could be suggested that the phytocostituent and antioxidant potential expressed by Annona muricata in hydroethanolic leaf extract could be effectively utilized for conducting further studies on identification of bioactive compounds for phytopharmacological application.

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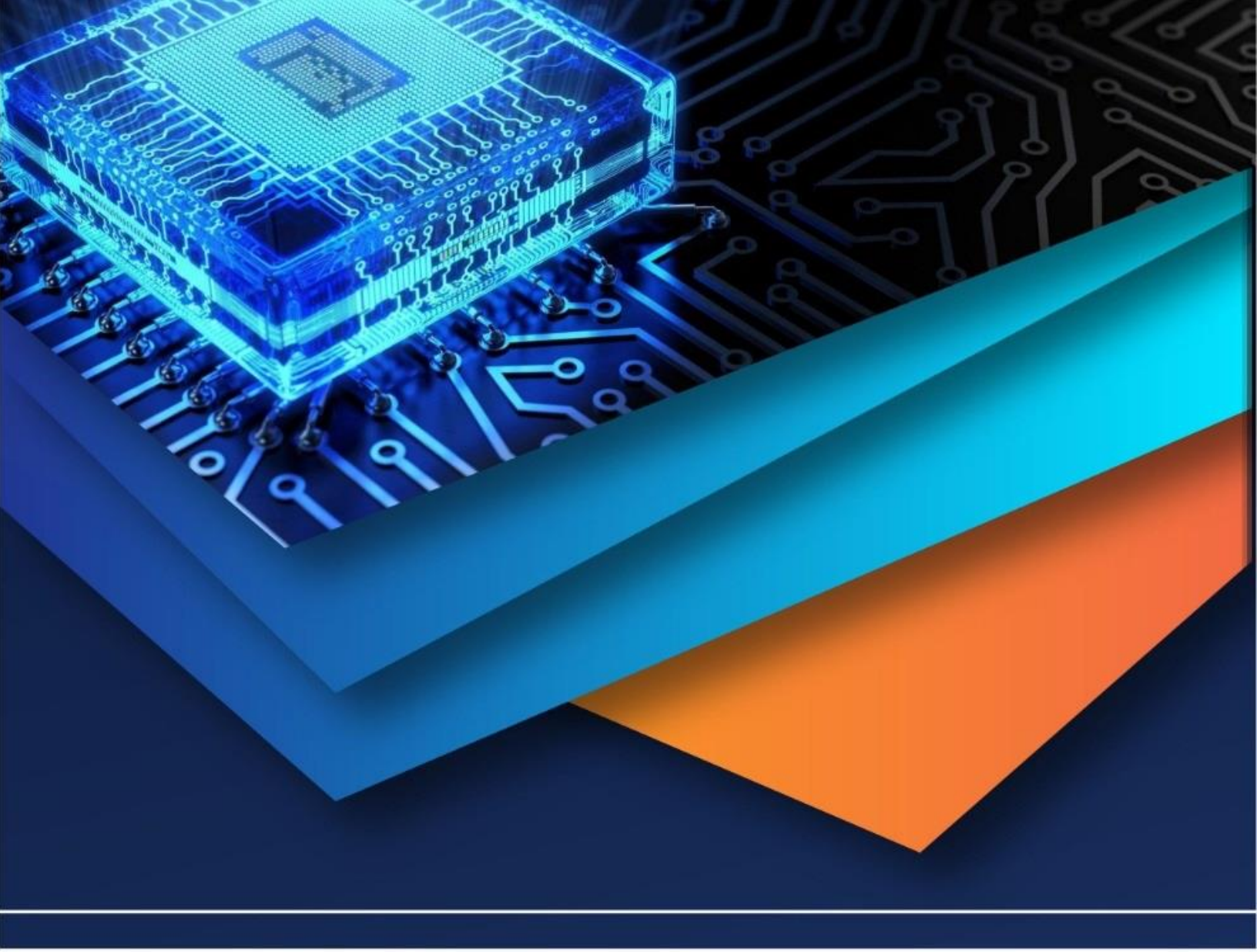

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