Identification of Some Annona Muricata L.(Soursop) Components and Their Antioxidant Effects in Rats

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ABSTRACT:

BACKGROUND:

Oxidative stress defines that, the level of Reactive Oxygen Species (ROS) exists in excess of antioxidant defenses. This imbalance in the redox milieu results in a switch from ROS-stimulated ambient signaling processes to ROS-mediated pathophysiological consequences. Oxidative stress has been implicated in the installation and progression of several degenerative diseases via DNA mutation, protein oxidation and lipid peroxidation. Therefore, possible use of soursop fruit extract to protect brain against the Lipid peroxidation.

OBJECTIVE:

The present study was undertaken to evaluate the potential of Soursop *(Annona Muricata L.)* against the DPPH Free Radical Scavenging System and Lipid peroxidation.

METHODS:

Phytochemical screening was carried out with fruit extract of A. muricata for the detection of various phytochemicals. The extract was tested for the presence of glycosides, proteins, saponins, tannins, phenolic compounds , alkaloids , flavonoids, steroids and vitamine C using the standard procedures and then DPPH radical was estimated according to the method of Blois and Lipid peroxidation was estimated according to the method of Rajakumar.

RESULTS:

The study showed that the Soursop (Annona Muricata L.) in the fruit extract contain : glycosides , proteins, saponins ,tannins ,phenolic compounds, flavonoids , alkaloids , steroids and vitamine C . Fruit extract were found effective in scavenging DPPH (78,6%) in concentration (250µl/ml), as well as inhibiting the lipid peroxidation (16.2%).

CONCLUSION:

The results suggest that Soursop (Annona Muricata L.) treatment protects the rat brain against lipid peroxidation and DPPH free radical scavenging.

KEY WORDS : soursop, phytochemical ,DPPH, lipid peroxidation

INTRODUCTION:

Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2^{-\cdot}$) and hydroxyl radicals ($OH^{\cdot}$) as well as non-free radical species such as hydrogen peroxide (H_2O_2) [(1)] .These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations [(2)].Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules [(3)] .The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS [(4)].Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors [(5)] .The natural antioxidant mechanisms maybe insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important [(6)].The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases [(1)].Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired

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widespread screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases [7].

Annona muricata L., a member of the Annonaceae family, is a widely distributed plant in Central and South America and tropical countries [8] .Also known as soursop and graviola, this small tropical tree plant has long been cultivated by native peoples, due to its extensive applications in folk medicine and heart-shaped, edible fruits [9] .The lanceolate dark green leaves of *A. muricata* are traditionally used as an antispasmodic nervine for heart conditions and as a sedative. In addition, the leaves are applied to treat asthma, cough, fever, headache, hypertension, and toothache [10].The leaves of *A. muricata* have been found to possess significant antioxidant effects, assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, ferric reducing antioxidant power, and hydroxyl-scavenging activity techniques in animal models [11].In addition, the leaves demonstrated a notable protective effect against acute and chronic inflammations in rats, through suppression of proinflammatory cytokines [12] .Previous studies have shown that the main chemical constituents in *A. muricata* are annonaceous acetogenins, alkaloids, and essential oils. Due to the significant antioxidant and anti-inflammatory features of *A. muricata* leaves, this plant may be a promising candidate for antiulcer agents.

MATERIALS AND METHODS:

Collection of samples:

The *(Annona Muricata L.)* were collected from market of Baghdad, Iraq. The fruit were transported to the laboratory biochemistry in department of chemistry /College of Science /Al-Mustansiryih University, washed, cleaned to remove all traces of dust and insects then Squeezed , filtrate,dried,weighed and placed in airtight bottles and stored to be used for extraction.

Chemical detection of the plant components:

The chemical components of the prepared fruit extract were detected using different tests as shown in Table .1.They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, flavonoids , proteins ,steroids and Vitamine C [13] .

DPPH Free Radical Scavenging System:

The effect of plant extracts on(2 diphenyl 2 picryl hydrazyl hydrate) DPPH radical was estimated according to the method of Blois. The absorbance of the resulting solution was measured spectro photometrically at 520 nm, Results were expressed as percentage inhibition of DPPH by comparing with blank [14].

scavenging effect (%) = (Ac-At)/Ac ×100

Lipid peroxidation:

The brain isolated from healthy albino rat (200-280 gm) was used as lipid source. Brain homogenate (10% w/v) was prepared in 150 mM KCl and centrifuged at 800 g for 10 minutes. The supernatant was collected and used immediately to study *in-vitro* lipid peroxidation. Briefly, the reaction mixture contained 0.3 ml of brain homogenate, KCl (100)M), ascorbic acid (100)M), ferricchloride (100)M), 0.5 ml of graded concentrations of extracts and final volume was made with buffer. After incubating at room temperature for 20 minutes, 1.0 ml of thiobarbituric acid-trichloroacetic acid (TBATCA) reagent was added. The resulted mixtures were heated at 800C for 20 minutes, cool and centrifuged for 10 minutes at 1000 rpm and by using a digital UV/VIS spectrophotometer recorded the absorbance at 532 nm. Control and standard (curcumin 10)M) were carried out at similar manner. Percentage inhibition of thiobarbituric acid reactive substance (TBARS) formation by extract/standard drug (curcumin) was calculated by comparing with control. All experiments were carried out in triplicate and results are the means of one such individual experiment. Percentage inhibition of lipid peroxidation by test compound [15]:

% inhibition = (Ac-At)/Ac ×100

RESULTS AND DISCUSSION:

The results in Table.1, showed that the extract gave positive tests for glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids , flavonoids, steroids and vitamine C .Similar results are also obtained by other studies.

Table 1: **Chemical components analysis for fruit extract of Soursop.**

Components	Reagents	Note	Result fruit extract
Glycosides	Iodine test Molish test Benedict test	Blue ppt. Violet ring Orange ppt.	Ve+ Ve+ Ve+
Proteins	Folin-Ciocalteau reagent	Blue color	Ve+
Saponins	Fast stirring Mercuric Chloride	Dense foam for long time White ppt.	Ve + Ve +
Phenolic compounds	Aqueous%1 Ferric chloride	Green ppt.	Ve+
Tannins	Aqueous%1 Ferric chloride Lead acetate%1	Green ppt. Preface yellow ppt.	Ve+ Ve+
Flavonoids	aqueous%1 Ferric chloride Ethanol hydroxide alcohol	Green ppt. Yellow ppt.	Ve+ Ve+
Alkaloids	Mayer's reagent Wagner reagent Picric acid	white ppt. Brown ppt. Yellow ppt.	Ve+ Ve+ Ve+
Steroids	Libermann-burchar Libermann's reagent	Green ppt. Blue color	Ve+ Ve+
Test for Fats and Oils	Solubility test		Ve+
Test for Vitamine C	Ascorbic acid	Yellow ppt	Ve+

(Table2) indicated that fruit extract of soursop has scavenged the DPPH stable free radicals in a concentration of 50,100,150 .200 and 250 µl/ml with their percentage inhibition

Table 2: DPPH free radical scavenging activity of fruit extract of Soursop.

Conc. extract (µg/ml)	scavenging effect (%)
50	17.6
100	36.9
150	53.5
200	67.1
250	78.6

As shown in Table 3: The amount of thiobarbituric acid reactive substance (TBARS) was calculated and percentage inhibition of TBARS formed was compared with control and standard drug (curcumin). The aqueous extracts of *Annona muricata L.* (50,100,150.200,250µl/ml) inhibited.

Table 3:Effect fruit extract of Soursop on Lipid peroxidation.

Conc. extract µg/ml	inhibition (%)
50	1.9
100	4.2
150	7.8
200	12.6
250	16.2

Virtually all plants have one or more phytochemical resident in their leaf, stem, root, fruit and flowers. Fruit extract of *A. muricata* contains phytochemicals including for glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids , flavonoids, steroids and vitamine C which are known to exhibit medicinal as well as physiological activities. Flavonoid are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be anti microbial substances

against wide array of microorganisms invitro [16]. They are also effective antioxidants and show strong anti-cancer activities [17]. The presence of these phytochemicals in *A. muricata* could be contributory to its antioxidant activity observed in this investigation. In the present experiment the order of increasing relative abundance of these phytochemical in the fruit extract of *A. muricata* is for glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids , flavonoids, steroids and vitamine C (Table 1).

Traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radical and reactive oxygen species can be involved in a high number of diseases [18]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

Antioxidants (free radical scavengers) are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system [19]. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body also relies on external sources, primarily the diet,to obtain the rest of the antioxidants it needs [20]. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants [21].

The scavenging activity of *Annona Muricata* extract was increases as the concentration was increased as shown Table 2 .this is in accordance with results of sharma and Bhat [22]. Moreover,the percentage of inhibition of Lipid peroxidation by different concentration of *Annona Muricata* extract as shown in Table 3 . certainly the incubation of *Annona Muricata* extract with brain homogenate reduced the Lipid peroxidation at large extent which indicate the defensive effect of soursop against Lipid peroxidation and TBARS formation as reported by Olakunle. [23]

CONCLUSION:

The present study confirm that the fruit extracts of soursop *(Annona Muricata L.)* posses *in vitro* antioxidant activity due of its content of glycosides , tannins , saponins ,proteins , phenolic compounds ,alkaloids , flavonoids, steroids and vitamine C .

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