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In-Vitro Anti-Oxidant Property Evaluation of Different Extract of *Artocarpus Chaplasha* Bark

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ABSTRACT

This investigation is made upon the plant *Artocarpus chaplasha*, the bark of it, to find out its anti-oxidant property. The anti-oxidant property of this plant part was investigated using different solvent for extraction, such as ethanol, ethyl acetate, methanol and chloroform. Different types extract is used to see different extent of result as many phytochemicals are readily extracted in ethanol but not in methanol, and so on. In case of anti-oxidant the scavenging power (IC_{50}) of DPPH radical was 55.15, 163.10, 166.94 and 407.875µg/ml for aforementioned extracts respectively. By the time IC_{50} ascorbic acid has 47.19µg/ml. There's also test for total phenolic and flavonoid content was carried out and the ressult for each was 3.63, 61.97, 2.15 and 3.18mg/gm for each extracts respectively in terms of phenolic content. Total flavonoid content was 116.17, 121.51, 82.33 and 63.67mg/gm for each different extracts respectively.

Keywords: IC_{50}, *A. chaplasha*, scavenging, reducing power, anti-oxidant

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INTRODUCTION

Plants are yet a good and dependable source of herbal and traditional medicine, as well as modern medicine also. Herbal medicine has been revealed with its valid utility and greate acceptability to all. This type of medicine is yet common and useful among more than 50% rural and tribal pepole by any means arround the world. As a thired world country, Bangladesh have great problem of disease management, which a big issue of concern. The knowladge about plant activity and their identification will provide aid to those who are dwellers of third world country as well as will reduce their cost in medicine. As a part of this we have studied the anti-oxidant property of *A. chaplasha* (family- Moraceae), as nautral anti-oxidants are more potent, available, economic than synthetic anti-oxidant.

Human begins need oxygen for their living and also need anti-oxidative agent for controlling oxidation. It is to be said that anti-oxidants are used to protect human begins from different ill effects due to increased production of reactive oxygen species (ROS) as a result of exposure to pollutants. The body has its natural mechanism to interact with oxidative species (ROS), but sometime it's not capable to fight strongly when they need anti-oxidant, most of time supplied through food (exogenous anti-oxidant) [1]. During the process of oxygen utilization about 5% of oxygen converts to its univalent derivative free radicals [2] like superoxide, peroxide etc. All these are known as reactive oxidative species (ROS), which attack cells of the body and each cell have face more than 1000 species of ROS per second [3]. Free radicals are always involved in different kind of degenerative diseases [4] like diabetes, liver damage, naphrotoxicity, cancer, inflammation and in the process of aging [5]. Plants that have enriched phytochemistry along with anti-oxidant significance are needed to cure disease like cancer [6]. The efficacy of plant extract has long been established and many more plants are on the way to be established, those plants that are found to have phenols, flavonoids, terpinoids, vitamins are most promising to have anti-oxidant property [7]. Among which that are found having in vitro anti-oxidant and vitamin A, E or C are extremely suggested for in vivo testing and further isolation processing [8]. Anti-oxidants are useful as anti-aging and prevention and curing of many degenerative diseases, for this purpose the bark of *A. chaplasha* was selected to evaluate its anti-oxidative power.

MATERIALS AND METHODS

Collection and Extraction

The plant part is available in wild zone of Bangladesh and was collected from Chittagong hill tracts. It was identified by renowned taxonomist of The University of Chittagong, Dr Shaikh

Bokhtear Uddin, Assistant Professor of Department of Botany.

It was extracted using cold extraction methods, which is most popular and cost effective. The plant after identification was dried for around a week and then brought for grinding to small pieces. The freshly macerated plant were then dissolved in methanol for primary extraction process.

After primary extraction followed by drying of liquid extract and preservation further extraction was made with the same plant. The methanol extract was fractioned in different solvent using common methods of fractional distillation [9].

Assay for scavenging power

DPPH (1, 1-Diphenyl-2-picrylhydrazyl radical) is a stable free radial that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. The radical scavenging potential of the sample was determined by measuring the decrease in absorbance due to DPPH at 517nm, representing the formation of its reduced form, 1, 1-Diphenyl-1-2-picrylhydrazine (DPPH), which was yellow in color, because of the odd electron, the purple colored methanolic solution shows a strong absorption band at 517nm[10].

Procedure

0.1ml of each cocentration of extract (10, 50, 100, 300 and 500µg/ml) was added to 3ml of 0.004% methanol solution of DPPH. The mixture was kept in dark place for proper reaction, as the reaction is light sensitive. After 30min, absorbance of the resulting solution was measured at specified wavelength. The percentage was calculated using formula following, where A_0 is absorbance of control and A_1 is ansorbance of test.

$$\%SCV = \frac{A_0 - A_1}{A_0} \times 100$$

Total phenolic content

0.5ml of plant extract at concentration of 200µg/ml was taken in three different test tubes for taking mean of absorbance. Ascorbic acid was taken as standard and at different concentration as, 10, 50, 100, 200µg/ml for calibration curve. 2.5ml Folin-Coicalteu (10 times dilute in water) and 2.5ml sodium carbonate (7.5%) was added to each test tube. All test tubes were incubated for 20min at 25^0C. Absorbance was taken at 760nm and calculation was carried out using following equation [11]. This method was described by Singleton *et al.* (1977).

$$C = \frac{c \times v}{m}$$

Where, C = total phenolic content, c = conc. of ascorbic acid from calibration curve, v= volume of extract and m = weight of plant extract

Flavonoid content

Total flavonoid content of different extract of *Artocarpus chaplasha* was determined by using aluminum chloride colorimetric method. 1ml of plant extract was taken in test tube where the extract was mixed with 200µl of 10% aluminum chloride, 3ml methanol 200µl of 1M potassium acetate and distilled water to make 6ml solution. The whole solution was allowed to stand for about 30min at room temperature, for accomplishing necessary reaction. Then at 420nm UV-spectrophotometric absorbance was noted and the total flavonoid content was calculated using Quercetin equivalency and following equation-

$$C = \frac{c \times v}{m}$$

Where, C = total phenolic content, c = conc. of quercetin from calibration curve, v= volume of extract and m = weight of plant extract

Reducing power capacity

In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reluctant such as antioxidant substances in the samples causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700nm of UV-spectrophotometric absorbance [12].

$$Fe^{3+} - ferricyanide + e \rightarrow Fe^{2+} - ferricyanide$$

The extract of testing was treated with 2.5ml potassium buffer (0.2M), 2.5ml potassium ferricyanide (1%) then heated for 20 mins at 50^0C. After that it was mixed with 2.5ml trichloro acetic acid (10%), solution was centrifuged for 10min to obtain supernatant from which 2.5ml was withdrawn and mixed with equal amount water followed by 0.5ml ferric chloride solution.

RESULTS AND DISCUSSION

In the finding section different fraction of *Artocarpus chaplasha* bark have promising result of anti-oxidant property. There are several methods to find out this property. There are multiple compounds may found in any fraction of a crude extract. They also may have variety in chemical nature such as polarity, chirality, and isomerism and so on, that can have effect on their anti-oxidative property. Depending on this we need to know the phenolic and flavonoid content that influence the anti-oxidant property. Phenolic and flavonoid contents were calculated for from ascorbic acid calibration curve and it's found that phenolic content of methanolic fraction is poor

while ethyl acetate fraction got both phenolic and flavonoid content more.

The scavenging power was estimated through DPPH testing method it is most common and considerable easy as well as time relaxing method. Result obtained by this method relatively good. 1, 1-Diphenyl-2-picrylhydrazyl radical is scavenged by anti-oxidant compound. Every anti-oxidant compound doesn't have likely scavenging power, as they vary their power can be measured through this testing at 517nm UV-spectrophotometric absorbance, therefore among other use of 1,1-Diphenyl-2-picrylhydrazyl one is in anti-oxidant assay[13].

Table 1: The table below represents the 1, 1-diphenyl-2-picrylhydrazyl radical scavenging capacity of different extract of *A. chaplasha* bark.

Concentrations (µg/ml)	Percent of scavenging			
	Ethanol	Methanol	Ethyl acetate	Chloroform
10	32.16	17.54	45.19	20.83
50	47.14	33.01	47.14	29.96
100	54.32	45.92	60.41	34.84
300	67.48	54.32	65.04	40.44
500	80.99	66.14	83.56	55.54

Table 2: The median inhibition concentration of different extract of *A. chaplasha* in comparison to reasonable standard compound is-

Test material	Extracts	IC_{50} (µg/ml)
Artocarpus chaplasha	Ethanolic	185.38
	Methanolic	293.24
	Ethyl acetate	158.27
	Chloroform	407.88
Ascorbic acid		47.19

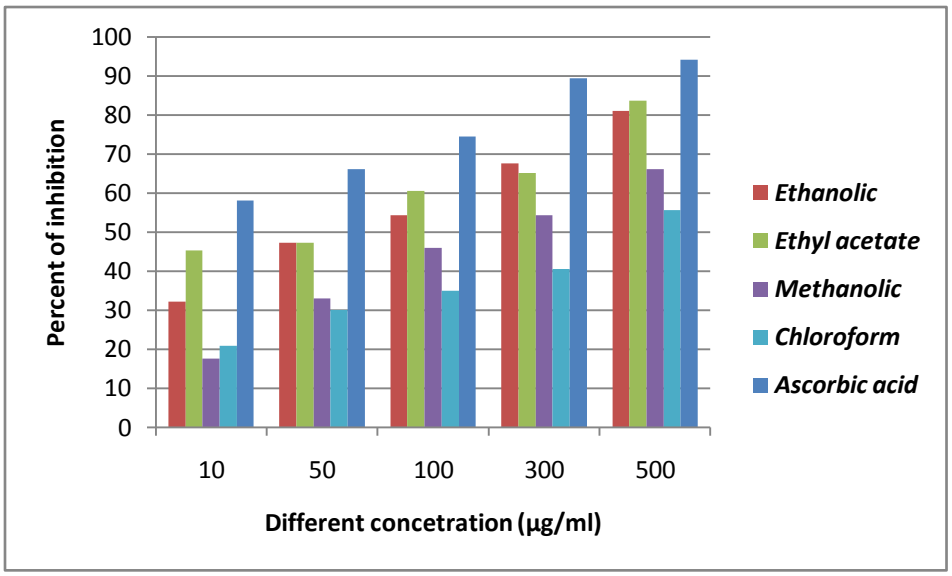

Figure 1: Scavenging activity of different fraction of *A. chaplasha* bark on DPPH radical along with comparable standard (ascorbic acid).

Reducing power

Reducing power of the crude extract of *A. chaplasha* was monitored and concluded by the transformation of Fe^{3+} to Fe^{2+}. The reducing power of tested sample was found concentration dependent and presented graphically in following figure (figure 2).

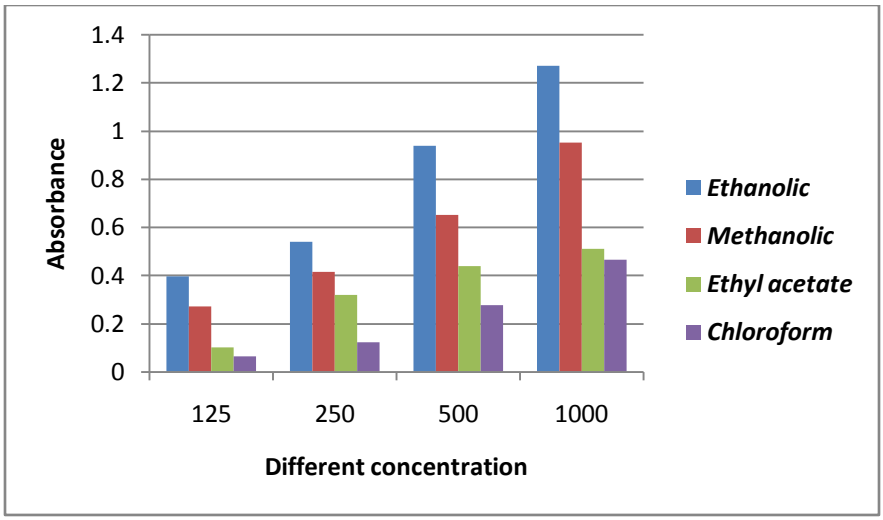

Figure 2: Reducing power capacity of *A. chaplasha* in treatment with ferric chloride radical, absorbance against different concentration.

Form recent data of various research it's found that anti-oxidants are essential to reduce or prevent several diseases, acute or chronic. Peoples of all ages, classes are suggested to consume fruits and vegetables much than synthetic foods, as they have natural anti-oxidant activity [14]. In aerobic condition animals need oxygen for living, free radicals (reactive oxidative species) are also consumed at same time in most cases. This free radicals cause several body malfunction as body immunity is compromised at their severe attack. Such kind of disease condition include cancer as there is many abnormal proteins form leading to malignancy, other diseases include gastritis, arthritis, CNS abnormality etc. Anti-oxidants reduce such kind of body functioning hindrance by interrupting free radicals [15]

From the experimental literature it is found that the plant extract has enrichment in phenolic and flavonoid content. It may be its major content of natural chemical resources and that's why causing anti-oxidant property, seems that phenolic or flavonoids are primary reason for such kind of activity. These compounds have record of broad spectrum action as anti-oxidant and they are originating for such reason from long period of time. Anti-oxidant activities of phenolics are caused by their donation of hydrogen atoms to free radical scavenging action. They possess an ideal structure for scavenging property [16].

DPPH is an established and well known free radical available commercially. Any compound or

candidates prove itself in DPPH scavenging assay would provide a promising result in in-vivo study. It was reported by Prasad *et al.* (2005) and Zhao *et al.* (2006) that phenolic and flavonoid reduces DPPH, enhancing scavenging power through their hydrogen donating ability [17, 18]. The result obtained by the investigation (table-1 & 2, figure 1) revealed that the scavenging power of *A. chaplasha* has very good action and attributor was may be hydrogen donating ability.

Reducing power is generally associated with the presence of reductones, which break down free radical chain by donating hydrogen atom, thus show anti-oxidant property [19]. In this assay ferricyanide having Fe^{3+} reduced to Fe^{2+}, by visual measurement it has seen navy blue color and final conformation had been confirmed by measuring at 700nm spectrophotometric absorbance. The reducing power of *A. chaplasha* may be due to its di or monohydroxyl substitute in the aromatic ring system, which probably present in it [20].

This experiment illustrate that the different fraction of *A. chaplasha* bark possesses different level of anti-oxidant action and are of promising. But among the entire fraction studied the ethyl acetate has the most effective and not decline able result. These also to be remember that this work is first time with this plant part. However, future study is needed with this to identify and isolation of the exact principle ingredient responsible for such kind of activity.

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