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Research Article

FREE RADICAL SCAVENGING ACTIVITIES OF *GARCINIA XANTHOCHYMUS* HOOK. F. AND *GARCINIA LANCEAEFOLIA* ROXB. USING VARIOUS *IN VITRO* ASSAY MODELS

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ABSTRACT

Objective: The present research work has highlighted the free radical scavenging activities of methanolic pericarp extract of *Garcinia xanthochymus* (MPEGX) and *Garcinia lanceifolia* (MPEGL) by using various *in vitro* assay models.

Methods: The present study was carried out by different methods such as 2,2/diphenyl-1-picrylhydrazine (DPPH), 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Nitride oxide, and total iron reducing power. Further, total phenolic contents and total flavonoids contents of the both crude extracts were also determined.

Result: The results revealed that MPEGX exhibited a high amount of phenolic contents and also showed the higher free radical scavenging activities in ABTS, Nitride oxide, and total iron reducing power. Similarly, the MPEGL contained high amounts of flavonoids contents and exhibited higher activity in DPPH model. From the result, it is suggested that the free radical scavenging activities of both the plant extract was directly co-related to both phenolic and flavonoids contents.

Conclusion: Due to significant free radical activities of both plant extracts, it is justified that these plants have a significant amount of total antioxidant compounds which has tremendous therapeutic potential.

Keywords: Antioxidant, Free radical scavenging, *Garcinia xanthochymus, Garcinia lanceifolia*, Pericarp.

INTRODUCTION

Free radicals are usually produced inside the mammalian body due to normal biochemical reactions. They sometimes act as factor of various human diseases. Reactive oxygen species (ROS), which donate electron or hydrogen atom to other substances for stability has directly affected on cellular function and structure. Human body creates many defense mechanisms for removal or neutralized the ROS, including antioxidant enzymes and non-enzymatic mechanisms. The antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase play a critical role in neutralizing free radicals and preventing the cells from different cellular injuries [1]. However, due to heavy exposure to external environmental pollutants such as smoke, UV radiation, pesticides, the self defense mechanism is insufficient to remove the excessive free radicals that cause the cellular stress, subsequently damage the function, and structure of cell membrane leading to generation of disorder such as Alzheimer's diseases, liver injury, cardiovascular diseases, atherosclerosis, diabetes, rheumatoid arthritis, etc., [2-4]. Therefore, supplementation of phytochemical which has the antioxidant properties has become a considerable attention. Now-a-day, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate consumption is under strict scanning as these causes serious diseases such as liver damage and carcinogenesis [5,6].

The epidemiological studies have shown that fruits and vegetables contain a higher nutritive value and also rich in antioxidants [7]. Therefore, considerable attention has been given to the natural antioxidant products by researchers.

Garcinia L. Belongs to the family Clusiaceae distributed throughout in tropical regions of the world. The genus has about 200 native to South Asia, ranging from southern parts of the Thailand, and peninsular Malaysia to Indonesia [8,9].

In India, 35 species of *Garcinia* L. were reported by Maheswari [10]. Out of 35 species, 9 species were found in undivided Assam and 8 species were reported in Sonitpur district of Assam [11,12]. *Garcinia* L. commonly known as "Thekera" in Assam, usually uses traditionally for the cure of several diseases such as dysentery, after childbirth medication, and fever [13]. Therefore, the present study was carried out to evaluate antioxidant properties of the fruit pericarp extract of *Garcinia xanthochymus* and *Garcinia lanceifolia* by various *in vitro* models.

MATERIALS AND METHODS

Collection of plant

The fruit of *G. lanceifolia* Roxb and *G. xanthochymus* Hook. f. were collected from the homestead garden and wild collected from the upper Assam between October 2013 to May 2014.

Identification and confirmation of plant

The collected plants were identified according to Kanjilal *et al.* [11] at the Department of Life sciences, Dibrugarh University, Assam, India, and sample voucher were submitted to the same.

Preparation of plant extract

Small pieces of fruit pericarp collected samples dried under hot air oven by maintaining the temperature between 35°C to 40°C. The oven dried samples were then powdered and 50 g of each sample were immersed in 100 ml of 80% methanol. The flasks were incubated in a shaker for 24 hrs (at 25°C) and extracts were filtered thought Whatman No. 1 filter paper. The collected extracts were dried under vacuum distillation and extract were kept in a refrigerator at 4°C for further analysis.

Preparation of test and standard solution

The pericarp extract of experimental *Garcinia* species and standard phenolic (gallic acid), standard flavonoids (rutin), and standard antioxidant (ascorbic acid) were dissolved separately in distilled

dimethyl sulfoxide (DMSO) in different concentration and used for further study.

Estimation of total phenolic content

Half ml of each plant extract (1000 µg/ml) was mixed with the 2.5 ml of Folin–Ciocalteu reagent (diluted 1:10 ratio with distilled water) and 1.5 ml of sodium carbonate (20% w/v). The mixtures were shaken thoroughly by using vortex and made up to volume 10 ml with double distilled water. The mixtures were then kept for 90 minutes. The absorbance was measured at 760 nm using UV-spectrophotometer. Different concentration (10-50 µg/ml) of gallic acid was used for the preparation of the standard curve. The total phenol content was expressed in gallic acid equivalent in mg/g of plant extract [14].

Estimation of total flavonoids content

Half ml of each plant extract (1000 µg/ml) were mixed with 1.5 ml of methanol (75% v/v), 0.1 ml (1 M) of potassium acetate, and keep for 1 minute. After 1 minute incubation, add 0.1 ml (10% w/v) aluminium chloride and made up the volume 3 ml with double distilled water, and kept the mixture for 30 minutes in dry and shady place. The absorbance was measured at 415 nm. Rutin was used as standard flavonoids for the preparation of calibration curve and total flavonoids were expressed as rutin equivalent in mg/g of plant extract [15].

2,2/diphenyl-1-picrylhydrazine (DPPH) radical scavenging assay

DPPH is a stable free radical which is usually converted into 1,1 dihydroxyl 2-picrylhydration whenever, it reacts with antioxidant and the degree of conversion can be measured by spectrophotometric method. Briefly, 2 ml of different concentration of plant extract and different concentration of standard solution (Ascorbic acid) were added to the freshly prepared 2 ml of DPPH solution (0.2 mM). After incubation of 30 minutes in the dark place, absorbance was taken in 517 nm by using UV-spectrophotometer [16]. The experiment was performed in triplicate and percentage of inhibition was calculated by following a formula.

$$\text{Percentage of inhibition } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100\%$$

2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphanic acid (ABTS) radical scavenging assay

The ABTS radical scavenging assay was carried out by the method of Re *et al.* [17], with minor modification. The ABTS radical was formed by the oxidation of ABTS with potassium per sulfate. The ABTS cation solution was prepared by mixing of 5 mL ABTS (7 mm) with 100 µl of potassium per-sulfate and incubated for 12 hrs. For preparation of working solution, a necessary amount of previous solution was added to the phosphate buffer saline solution till the absorbance was 0.70±0.001 at 734 nm. The fleshly prepared working solution was then kept in the dark for 30 minutes. A volume of 1 ml of different concentrations of plant extract was mixed with 1 ml of working solution and mixed thoroughly; kept in the dark for 10 minutes. The absorbance was measured at 734 nm. The percentages of inhibition were calculated by above-mentioned formula.

Nitrite oxide (NO) radical inhibition assay

NO was generated by dissolving the sodium nitroprusside in aqueous solution which was measured by Griess reaction. Scavengers of NO compete with oxygen to help reduce the production of nitride oxide [18]. The reaction (6 ml) mixer was prepared by mixing of sodium nitroprusside (10 mM, 4 ml), 1 ml of different concentration of sample or standard (dissolved in DMSO), and phosphate buffer saline (PBS, 1 ml PH 7.4) and kept the mixer for reaction at 25°C for 150 minutes. After the reaction, 0.5 ml of reaction mixer was removed and transferred to the Griess reagent and incubated for 30 minutes at diffused light. Griess reagent was prepared by mixing of sulfanilic acid (1 ml, 0.33% w/v) and napthylethylenediamine dihydrochloride (1ml, 0.1% w/v). The absorbance was measured at 540 nm [19]. Percentage of inhibition was calculated following formula,

$$\text{Percentage of inhibition } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100\%$$

Total iron reducing power assay

The reducing power total iron of both plant extracts were determined by the method of Atmani *et al.* [20] with slight modification. Briefly, 1 ml of plant extract (1 mg/ml) was mixed with 2.0 ml of phosphate buffer saline (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v).The mixture was incubated at 50°C for 20 minutes. After incubation, 2.5 ml of trichloroacetic acid (10% v/v) was added to the mixture and then the reaction mixture was centrifuged at 1000 RPM for 10 minutes. The upper layer of the mixer (2.5 ml) was removed and added to the double distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1% w/v) and mixed thoroughly by using vortex. The absorbance was measured at 700 nm. The higher absorbance result as higher reducing power [21].

RESULTS

Total phenol content and total flavonoids content

The results revealed the higher amount of total phenolic contents in MPEGX than MPEGL. On the other hand, MPEGL exhibited a high amount of total flavonoids contents compared to MPEGX. The results were expressed in both total phenolic and flavonoids contents in gallic acid equivalent (mg/g of extract) and rutin equivalent (mg/g extract), respectively. The summary of results was shown in Table 1.

DPPH and ABTS radical scavenging activities

The MPEGL exhibited higher free radical scavenging potentiality than the MPEGX in DPPH model, where at the ABTS model MPEGX exhibited higher radical scavenging potential. The IC_{50} value of ascorbic acid in DPPH model was less than IC_{50} values in both MPEGX and MPEGL. In ABTS model, the IC_{50} of ascorbic acid was higher than the MPEGX. DPPH and ABTS free radical scavenging activity of plant extracts were also estimated (Table 2).

Table 1: Estimated value of TPC and TFC

Serial number	Plant extract	TPC	TFC
		GAE mg/g dry extract	RE mg/g dry extract
1	MPEGX	219.23±0.15	63.50±2.12
2	MPEGL	120.34±2.99	128.99±6.13

TPC: Total phenolic content. TFC: Total flavonoids content, Values are mean±SEM of three replicates, MPEGX: Methanolic pericarp extract of *Garcinia xanthocymus*, MPEGL: Methanolic pericarp extract of *Garcinia lanceaefolia*, GAE: Gallic acid equivalent, RE: Rutin equivalent, SEM: Standard error mean

Table 2: Antioxidant activities of *G. xanthocymus* and *G. lanceaefolia*

Serial number	Sample/ standard	IC_{50} (µg/ml)		
		DPPH	ABTS	Nitride oxide
1	MPEGX	205.84±15.11	54.29±2.59	59.52±1.19
2	MPEGL	75.5±5.0	58.0±2.50	175.59±13.77
3	Ascorbic acid	49.5±1.00	67.62±0.99	262.93±12.60

Values are mean±SEM of three replicates, MPEGX: Methanolic pericarp extract of *Garcinia xanthocymus*, MPEGL: Methanolic pericarp extract of *Garcinia lanceaefolia*, SEM: Standard error mean, *G. lanceaefolia: Garcinia lanceaefolia, G. xanthocymus: Garcinia xanthocymus*, ABTS: 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfanic acid, DPPH: 2,2/diphenyl-1-picrylhydrazine

Nitride oxide scavenging assay

Both extracts showed a strong NO scavenging activity when compared with standard ascorbic acid. The IC_{50} value of both extracts was less than the ascorbic acid (Table 2).

Total iron reducing power assay

Fig. 1 showed the ability of both plant extracts to reduce the total iron, compared to the ascorbic acid. The higher absorbance means higher reducing power. The MPEGX showed the maximum absorbance than the MPEGL extract.

DISCUSSIONS

Oxygen is essential elements for the life, but has a deleterious effect on the human body under certain conditions. The most harmful effect of oxygen is the formation of active compounds known as ROS. ROS donates an electron to other substances and become reactive themselves. Many reactive compounds are free radicals, which have one or more unpaired electron rather than match pair. They are highly unstable and reactive. They cause damage to other molecules by extracting electrons from them in order to stay stable. Free radicals are continuously formed inside the human body because they are formed in both enzymatic and non-enzymatic reactions. Besides this, these are also continuously derived from each metabolic reaction because they are essential for detoxification, chemical signaling, and proper immune function. Usually, free radicals are regulated inside the human body by endogenous antioxidant system, but due to over production of free radical by non-enzymatic formation such as UV radiation, cigarette smoking, and other external environmental factors, body self-antioxidant enzymatic system is enable to protect the cells from the attack of free radicals and the chances of diseases such as Alzheimer's disease, Parkinson's disease, Liver disease, cardiovascular disorder even to cancer is increased [21,22]. Therefore, it is very essential for external supplemental of antioxidant with diet. Some non-enzymatic antioxidant such as carotenoids, ascorbic acid, phenolic, flavonoids, etc., can protect the human body by acting one or more mechanical like free radical scavenging, reducing activity, etc. These antioxidant compounds are usually occurring naturally; therefore, the discovery of natural antioxidant is a major research area now a day [23]. The free radical scavenging activities of MPEGX and MPEGL were investigated against various *in vitro* models. It is essential to test the extract in different *in vitro* models to prove the antioxidant properties because free radicals are staying in different chemical forms. Hence, selective above mentioned models used to screen.

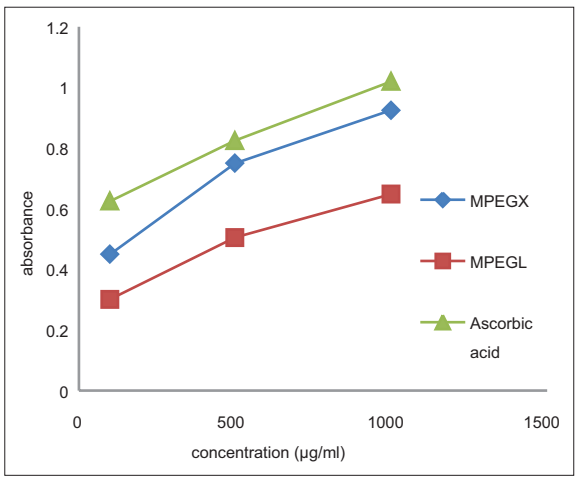

Fig. 1: Total iron reducing power of MPEGX and MPEGL compared with ascorbic acid, Values are mean±SEM of three replicates, MPEGX: Methanolic pericarp extract of *Garcinia xanthocymus*, MPEGL: Methanolic pericarp extract of *Garcinia lanceaefolia*, SEM: Standard error mean

The literature reviewed suggested that the total phenolic content constitute a major phenol containing group, which were served as a primary antioxidant or free radical scavenger. Flavonoids compounds are the most diverse group of natural compounds that has ability to scavenge the free radicals. From the present investigation, it was revealed that the total phenolic contents were more in the MPEGX and total flavonoids contents were high in MPEGL. Similarly, phenolic and flavonoids can scavenge the free radicals by donating a hydrogen atom to free radicals to make them to stable [24].

The DPPH is a stable free radical compound which forms the free radicals in solvents. The DPPH system is widely accepted tools to determine the free radical scavenging activities of antioxidants; it accepts an electron or hydrogen radical to become stable diamagnetic molecules [25]. The deductive capability of antioxidant in DPPH system can be determined by decreasing absorbance at 517 nm, experimentally. Sometimes it is easier to determine the DPPH radical scavenging of antioxidant by changing the purple color of DPPH to light yellow color. The experimental data of both plant extracts revealed that MPEGL has greater potential to remove free radical or has higher antioxidant properties than MPEGX, especially in DPPH model. From the results, it is observed that a dose-dependent relationship in DPPH model up to a saturated point. The flavonoids and phenolic compounds of MPEGL are probably responsible factors in the free radical scavenging ability [26].

The ABTS is relatively a recent free radical scavenging method, which produces more drastic radicals, often used for screening complex antioxidant from the mixture such as plant extract, biological beverage, and fluids [21]. The results showed that the MPEGX had high free radical scavenging properties in the ABTS model than MPEGL. From the results, it was also ensured that the ABTS radical scavenging activity was directly correlated with the total phenolic content of the plant extract.

Nitride oxide is produced continuously inside the human body; involved in the regulation of various metabolic processes. However, excess production of NO lead to several diseases such as inflammation, cancer, etc. NO is very unstable under aerobic conditions and usually react with oxygen to produce a stable product, i.e., nitrate and nitrite by producing intermediates such as NO_2, N_2O_4, and N_3O_4 [27]. These free radicals are responsible for alternating the functional behavior of a cellular component by changing the structure. Both plant extracts showed the best result in nitride, oxide model compared to the ascorbic acid. The nitride oxide free radical scavenging was higher in the MPEGX than MPEGL and ascorbic acid, respectively. The free radical scavenging principle of this model is that the total antioxidants present in plant extract competing with oxygen to react with nitride oxide and in this way inhibited the generation of anion and protect the cellular components from the attack of nitride oxide [21]. Nitride oxide was produced in this *vitro* model by incubation of sodium nitroprusside in phosphate buffer saline at 25°C.

Total iron reducing ability of the plant extracts can be measured by absorbance at 700 nm. Higher absorbance means the higher reducing power. Usually, reductive ability depends on the $Fe3^+$ transform to $Fe2^+$. The $Fe3^+$ reduction is often used for indicating the electron donor, i.e. antioxidant ability of plant exact and properties of phenolic antioxidant [28]. The reduced ability of plant extract is directly correlated with the present of reductions, which exhibit antioxidant properties by breaking of the free radical chain by donating a hydrogen atom [29]. The experimental data revealed that the MPEGX showed the high absorbance in different concentration than the MPEGL; however, absorbances of both extracts were less than the standard.

CONCLUSION

This biochemical test revealed that MPEGX is a good source of phenolic compounds, whereas MPEGL has a significant amount of both phenolic and flavonoids compounds. Therefore, both the plants showed distinctive results in various free radical scavenging properties

in vitro. It was also revealed that both the flavonoids and phenolic compounds have significant contribution in antioxidant properties. Therefore, MPEGX, which exhibits a high amount of phenolic contents high antioxidant properties *in vitro* models (*viz.*; ABTS, Nitride oxide, and total iron reducing power). On the other hand, the MPEGL which exhibits a high amount of flavonoids showed the high antioxidant property in DPPH model. Further studied is needed to isolate, purify, and identification of bioactive compounds from the both plants and study their antioxidant properties.

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