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Research Article

PHYTOCHEMICAL PROFILE AND *IN VITRO* ANTIOXIDANT ACTIVITY OF *GARCINIA GUMMI- GUTTA* (L) PEEL EXTRACTS

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ABSTRACT

Phytotherapy is considered as a complementary approach for preventing and treating simple disease, although well grounded in medical tradition, it often lacks proper scientific validation. *Garcinia gummi-gutta* (L.) Robson (family Guttiferae, also called Clusiaceae) is an evergreen tree of medium size. Fruit is the economically important part of the tree and its trade is at international level. The objective of the present study was to analyze the phytochemicals and antioxidant potential of *Garcinia gummi-gutta* peel extracts. Phytochemical analysis was carried out following standard procedures. *In vitro* antioxidant properties of the extract were analyzed by DPPH, OH radical scavenging and reducing power assays. From this analysis, methanolic peel extract was found to have more constituents compared to ethanolic and aqueous extracts. Also methanolic peel extract exhibited highest antioxidant and reducing power. The present study showed that among various extracts tested, the garcinia peel methanolic extract was found to possess notable antioxidant property.

Keywords: *Garcinia gummi-gutta,* Phytochemical, Antioxidant.

INTRODUCTION

Natural products are the source of synthetic and traditional herbal medicine. They are still practised as the primary health care system in many parts of the world[1]. In recent years, secondary plant phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[2]. Thus, it is anticipated that phytochemicals with adequate medicinal efficacy will be used for the treatment of various ailments[3]. Since time immemorial, various parts of plants have been used in the treatment and prevention of various diseases[4]. Free radicals play a crucial role in the pathogenesis of several human diseases and ageing. They are by products of various endogenous processes or a result of external factors, such as irradiation and xenobiotics. Antioxidants protect against free radicals and they are therefore essential in obtaining and preserving good health[5]. In recent years, much attention has been devoted to natural antioxidant and their association with health benefits. Plants are potential sources of natural antioxidants. It produces various anti oxidative compounds to counteract reactive oxygen species (ROS) in order to survive[6]. In order to identify the most potent antioxidants, the antioxidant profiles of numerous compounds are frequently compared. *Garcinia gummi-gutta* (L.) Robson (family Guttiferae, also called Clusiaceae) is an evergreen tree of medium size. It grows on the humid slopes along the wet evergreen forests of the Western Ghats, India and Srilanka. In India, population densities are highest in Uttara Kannada district, Karnataka, which is the northern part of the range of this species[7]. Fruit is the economically important part of the tree. The pulp of the fruit rind, is used in curries as a souring condiment, and is known to have antiseptic properties. Dried seeds yield kokum butter, rich in protein and fat. The oil is traditionally used for treating skin diseases. The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and tannins, etc. The active principles usually remain concentrated in the storage organs of the plants[8]. Considering all these facts, the present study was designed to investigate the presence of various phytochemicals, screening the antioxidant potential and free radical scavenging activity in the three different extracts of *Garcinia gummi-gutta* (L.) peel, a plant which evokes various therapeutic effects.

MATERIALS AND METHODS

Collection of Plant Material

The fruit of *Garcinia gummi-gutta* were collected from Coorg and were identified.

Preparation of Plant Extracts

Ethanol and Methanol extract

The fruit peel was dried in hot air oven at 40^{o}-50^{o}C for a week. The dried plant material was powdered using mixer grinder and subjected to soxhlet extraction with 99 % ethanol and methanol for 24 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

Aqueous extract

The peel powder was boiled in distilled water for 15-20 minutes, kept in room temperature overnight and filtered. The filtrate was evaporated to dryness in hot air oven and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

Phytochemical analysis of different Crude extracts

The extracts viz., Ethanolic Garcinia Peel (EGP), Methanolic Garcinia Peel (MGP) and Aqueous Garcinia Peel (AGP) were tested for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Following standard procedures were used[9-10].

Test for Steroids and Triterpenoids

Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Test for Glycosides
Keller Killiani Test

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Bromine Water Test

Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Test for Saponins
Foam Test

Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Alkaloids
Hager's Test

Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Flavonoids
Ferric Chloride Test

Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test

Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test

Test solution when treated with few drops of lead acetate (10 %) solution would result in the formation of yellow precipitate.

Test for Tannins
Gelatin Test

Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Test for Proteins
Biuret Test

Test solution was treated with 10 % sodium hydroxide solution and two drops of 0.1 % copper sulphate solution and observed for the formation of violet/pink color.

Test for Free Amino Acids
Ninhydrin Test

Test solution when boiled with 0.2 % solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Carbohydrate
Benedict's test

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Vitamin C
DNPH Test

Test solution was treated with Dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Antioxidant Activity
DPPH radical scavenging assay

Antioxidants react with DPPH, a stable free radical which is reduced to DPPH-H and as a consequence the absorbance is decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. The free radical scavenging capacity of the *G. gummigitta* peel ethanolic, methanolic and aqueous extracts was determined using DPPH according to the method of Blois[11]. DPPH solution (0.004 % w/v) was prepared freshly in 99 % ethanol and was added to sample solutions (1000 µg/ml) in ethanol. The mixture was allowed to stand at room temperature in dark for 20 minutes. Then the mixture was vortexed and the absorbance was read at 517 nm using a spectrophotometer. Ellagic acid was used as a reference standard. Control sample was Prepared, containing the same volume without any extract and 99 % ethanol was used as blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All tests were performed in duplicates. Percentage scavenging of the DPPH free radical was measured using the following equation, DPPH radical scavenging activity (%) = (Acontrol-ATest)/Acontrol X 100. Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the extracts or standard.

Hydroxyl radical scavenging assay

This was assayed as described by Elizabeth and Rao[12] with a slight modification. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction). The reaction mixture

contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); H_2O_2 (1.0 mM); ascorbic acid (100 µM) and 1000 µg/ml of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml of 2.8 % TCA, then 1 ml 1 % aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. Reaction mixture without test substances/extracts was used as control. All tests were performed in duplicates. Ellagic acid, a classical OH scavenger, was used as a positive control. Lower absorbance of the reaction mixture indicated higher OH radical scavenging activity. Percentage inhibition was evaluated by comparing the test and blank solutions. Percentage scavenging of the OH radical was measured using the following equation, OH radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}$ X 100. Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample or the extract.

Reducing power (FRAP) assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu[13] with a slight modification. 1000 µg/ml of the extracts (0.5 ml) were mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium hexacyanoferrate (1 %), followed by incubation at 50°C in a water bath for 20 min. After incubation, 0.5 ml of TCA (10 %) was added to terminate the reaction. The upper portion of the solution (1 ml) was mixed with 1 ml distilled water, and 0.1 ml $FeCl_3$ solution (0.1 %) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. All tests were performed in duplicates. A higher absorbance of the reaction mixture indicated greater reducing power. Ellagic acid was used as a positive control.

Statistical Analysis

All values were expressed as Mean ± SD of two measurements. Comparison between standard and extracts were performed by using One Way ANOVA using Tukey's test for multiple comparison. In all these tests, criterion for statistical significance was $P < 0.05$.

RESULTS

Phytochemical Analysis

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The various extracts of *Garcinia gummi-gutta* peel have revealed the presence of Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Carbohydrate and Vitamin C. Proteins, saponins, alkaloids and free amino acids were found to be absent in all the extracts. From this analysis, methanolic peel extract was found to have more constituents compared to ethanolic and aqueous extracts. The results of preliminary phytochemical analysis are shown in Table 1.

Antioxidant Studies

DPPH Radical Scavenging Assay

DPPH is one of the free radicals, widely used for assessing preliminary radical scavenging activity of the plant extracts. The radical scavenging effect of ethanol, methanol and aqueous extracts of Garcinia peel is summarized in Figure 1. The percentage of radical scavenging property of EGP, MGP and AGP and standard Ellagic acid in this assay were 72.55 ± 1.59, 90.03 ± 0.79, 57.33 ± 0.53 and 94.64 ± 1.72 at 1000 µg/ml respectively. In this assay, MGP exhibited highest radical scavenging potential similar to standard Ellagic acid with non-significant difference ($P > 0.05$), than EGP and AGP.

Hydroxyl radical scavenging assay

Scavenging of hydrogen peroxide by different extracts of *Garcinia gummi-gutta* peel is presented in Figure 2. The percentage of radical scavenging property of EGP, MGP and AGP and standard Ellagic acid in this assay were 33.13 ± 2.11, 69.25 ± 0.42, 21.94 ± 2.74 and 75.22 ± 1.26 respectively. The percentage of H_2O_2 scavenging activity of methanolic extract was found to be the highest among the three extracts with non significant difference with standard Ellagic acid ($P > 0.05$).

Ferric Reducing Antioxidant Power (FRAP) assay

The extracts exhibited potent antioxidant property by reducing power ability. Results of reducing power assay are shown in Figure 3. The EC_{50} value of EGP, MGP, AGP and EA in this assay were 328.95 ± 2.44, 218.96 ± 0.06, 254 ± 1.82 and 214.82 ± 4.69 µg/ml respectively. In this assay, MGP exhibited highest reducing power similar to standard Ellagic acid with non-significant difference ($P > 0.05$) than EGP and AGP.

DISCUSSION

The present study investigated the phytochemicals and Antioxidant potential of various three different extracts of *G. gummi-gutta* peel. Due to the presence of phytochemical constituents, the medicinal plants are useful for healing as well as for curing of human diseases[14]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. Of the ten phytochemicals screened for, seven were found present in various solvent extracts. They are triterpenoids and steroids, glycosides, flavonoids, tannins, carbohydrates and vitamin C. In all, more phytochemicals was found to be present in methanolic peel extract than ethanolic and aqueous extract. Remarkably, glycosides, flavonoids, carbohydrates and vitamin C were present in methanolic peel extract. This suggests that the methanol peel extract offers a wider array of phytochemicals than the ethanol and aqueous extract. The result indicates that *Garcinia gummi-gutta* peel hold promises as source of pharmaceutically important phytochemicals. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti carcinogenic etc[15]. Reactive Oxygen Species has received considerable attention in the recent past, because of its role in several pathological conditions. DPPH radical is considered to be a model of lipophilic radical. In this mode, scavenging activity is attributed to hydrogen donating ability of antioxidants[16]. Although methanolic extract of *Garcinia gummi-gutta* peel possess good DPPH scavenging activity, it was evident that the extract could serve as free radical inhibitors or scavengers. Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity. Hence removing H_2O_2 is very important for the protection of living systems[17]. *Garcinia gummi-gutta* peel methanol extract scavenged hydrogen peroxide more significantly which may be attributed the presence of phytoconstituents that could donate electrons to hydrogen peroxidase, thereby neutralizing it into water.

Table 1: Qualitative Phytochemical Screening

Chemical tests	Peel extract		
	Ethanol	Aqueous	Methanol
I. Test for Triterpenoids and Steroids			
Liebermann Burchard Test	-	+	-
II. Test for Glycosides			
Keller Killiani Test	-	+	+
Bromine water	-	+	+
III. Test for Saponins			
Foam test	-	-	-
IV. Test for Alkaloids			
Hager's Test	-	-	-
V. Test for Flavonoids			
Ferric Chloride test	+	-	+
Alkaline reagent test	+	-	+
Lead Acetate Solution test	+	-	+
VI. Test for Tannins			
Gelatin Test	+	-	-
VII. Test for Proteins			
Biuret test	-	-	-
VIII. Test for Free amino acids			
Ninhydrin Test	-	-	-
IX. Test for Carbohydrates			
Benedict's Test	+	-	+
X. Test for Vitamin C			
DNPH test	-	-	+

'+' and '-'indicates the presence and absence of phytochemicals respectively

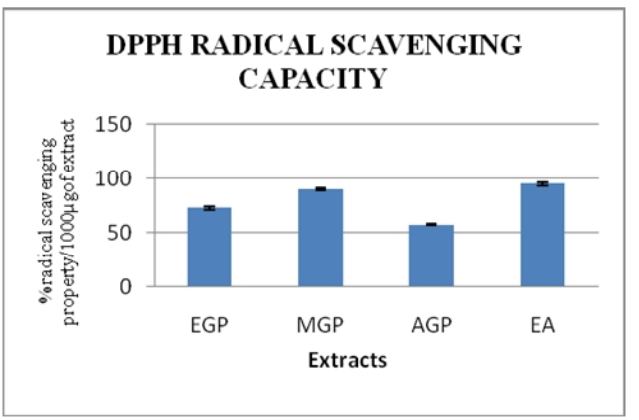

Figure 1: Relative percentage of radical scavenging activity for *G. gummi-gutta* peel extracts and standard Ellagic acid by DPPH method

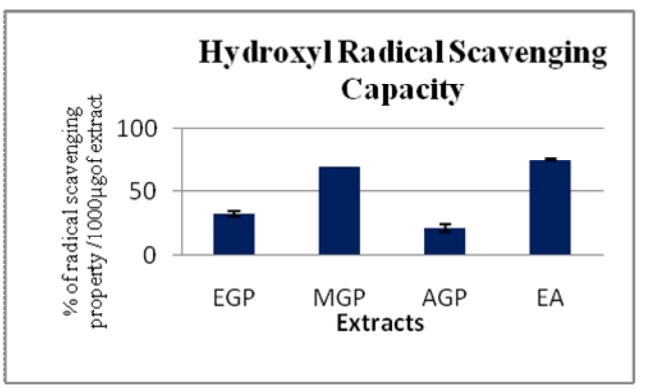

Figure 2: Relative percentage of OH radical scavenging activity for *G. gummi- gutta* peel extracts and standard Ellagic acid

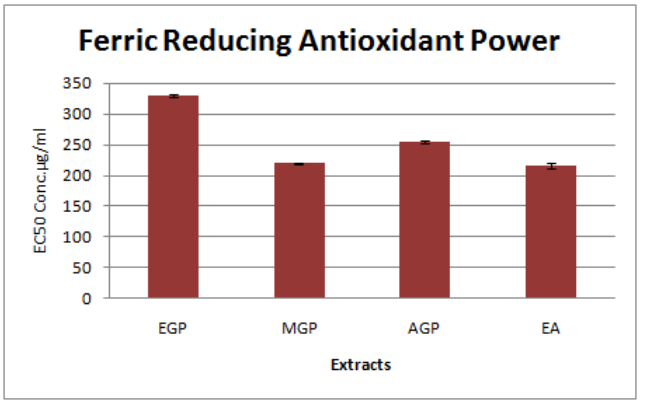

Figure 3: Ferric reducing antioxidant potential of *G. gummi-gutta* peel extracts and standard Ellagic acid by FRAP assay

A reducing power is an indicative of reducing agent having the availability of atoms which can donate electron and react with free radicals and then convert them into more stable metabolites and terminate the radical chain reaction[18]. Accordingly, *Garcinia gummi-gutta* peel extract might contain a sizable amount of reductants which may react with the free radicals to stabilize and terminate from free radical chain reaction. The decrease in the concentration of FRAP is a measure of the reducing potential of *Garcinia gummi-gutta* peel extract. However the methanol peel extract exhibited potent reducing ability similar to standard ellagic acid. It is reported that the phytochemical constituents directly influence the antioxidant properties of the plant extracts[19]. The antioxidant activity of plant extracts is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers[20]. The results suggest that the methanolic peel extract of *Garcinia gummi-gutta* showed higher antioxidant activity due to the presence of glycosides, flavonoids, carbohydrates and vitamin C.

CONCLUSION

This study suggests that the methanolic peel extract of *Garcinia gummi-gutta* possess more phytochemical constituents and highest free radical scavenging activity. The conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to reach a concrete conclusion about the findings of the present study. This evaluation might be useful for further studies to unravel novel treatment strategies for diseases associated with free radical or chemical induced tissue damage.

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