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EVALUATION OF *IN VITRO* ANTIOXIDANT PROPERTIES OF *GARCINIA BRASILIENSIS* LEAF EXTRACT

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ABSTRACT: The *in vitro* antioxidant activity of n-hexane, chloroform, ethyl acetate and alcoholic fraction of the total ethanolic extract of the leaves of *Garcinia brasiliensis* have been tested using various antioxidant model systems *viz.*, ferric reducing power assay and hydrogen peroxide scavenging assay. Ethyl acetate fraction is found to possess higher ferric reducing power and superoxide radical scavenging activity. The estimation of total phenolic and flavonoid contents of the various fractions are studied and the ethyl acetate fraction is found to possess high total phenolic and flavonoid content, thus correlating it with the antioxidant potential of the ethyl acetate fraction.

INTRODUCTION: Oxidative stress is a result of imbalance between the antioxidant defence system and the formation of reactive oxygen species (ROS). Oxidative damage may causes cell injury, death and exacerbate the development of several age related chronic diseases including cancer, Alzheimer's disease, Parkinson's disease and heart disease [1].

Antioxidants are the molecules of defence that quench these hazardous free radicals and reduce their potential to attack the cells, thereby protecting the cells. They neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition.

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This means that the antioxidant molecule becomes a free radical molecule from a non-free radical molecule, but the antioxidant molecule will usually be much less reactive free radical than the free radical neutralized. Antioxidants combat oxidation [2]. Protection against oxidative stress is one of the fundamental functions of the secondary metabolites in plants. This means that the plants in which the secondary metabolites is particularly developed will be rich in antioxidant substances and thus the conditions in which they grow or are cultivated have important effects on product quality [3].

Antioxidant properties of phenolics and flavonoids: Flavonoids are well known for their antioxidant activity. Antioxidants are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals (reactive oxygen species ROS). An imbalance between antioxidants and free radicals results in oxidative stress, which may lead to cellular damage [4].

Natural or phytochemical antioxidants such as phenolic acids, flavonoids and carotenoids are secondary metabolites in plants. They are amongst the antioxidants produced by plants for their sustenance. Recently, phenolics and flavonoids have been considered as great antioxidants and proved to be more effective than Vitamin C, E and carotenoids. The antioxidant properties of phenolic and flavonoid compounds are mediated by the following mechanisms: (1) scavenging radical species such as ROS/ reactive nitrogen species (RNS); (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production; (3) up regulating antioxidant defence. The reduction activity of phenolic and flavonoid compounds depends on the number of free hydroxyl groups in the molecular structure, which would be strengthened by steric hindrance [5].

Garcinol has high antioxidant activity in comparison with ascorbic acid and α-tocopherol. In addition, garcinol showed significant chelating activity and high scavenging activity against hydroxyl radicals. This was further investigated *in vivo* and it was shown that garcinol efficiently prevented the development of gastric injury and ulcer, in comparison with a positive control [6]. These findings are confirmed by ethnobotanical practice and traditional use of *G. brasiliensis*, which has been reported in Brazilian folk medicine to treat peptic ulcer [7].

The antioxidant properties of phenolic compounds are mainly because of their redox potential, which allow them to act as reducing agents, hydrogen donors, metal chelators and singlet oxygen quenchers [8]. It is known that the degree of glycosylation significantly affects the antioxidant properties of the compounds, for example, aglycones of quercetin and myricetin were more active than their glycosides.

The chemical structure of polyphenols gives them the ability to act as free radical scavengers. The type of compound, the degree of methoxylation and the number of hydroxyl groups are some of the parameters that determine the antioxidant activity. As for phenolic acids, the oxidation inhibition is related to the chelation of metal ions via the *ortho* dihydroxy phenolic structure, the scavenging of alkoxyl and peroxyl radicals, and the regeneration of α-tocopherol through reduction of the tocopheryl radical [9].

MATERIALS AND METHODS:

Collection of the leaves of *Garcinia brasiliensis:* The leaves of *Garcinia brasiliensis* were collected in the month of February. A voucher specimen is preserved at the Herbarium of School of Environmental Sciences, M.G University, Kottayam.

Extraction and Fractionation: [10] Extraction of the dried leaves of *Garcinia brasiliensis* were carried out using ethanol by hot extraction using the reflux condensation. The total ethanolic extract (TE) was then fractionated using solvents in the increasing order of polarity.

Estimation of total phenolic content: Total phenolic contents were estimated using Folin-Ciocalteau reagent based assay [11]. 1mL aliquots of 25, 50, 75 and 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The gram equivalents of gallic acid per 100g of the various fractions were obtained

Estimation of flavonoids: The total flavonoid content is estimated by Aluminium Chloride Colorimetric assay [12]. 1mL aliquots of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mcg/ml methanolic rutin solutions were used as standard for calibration curve. The gram equivalents of rutin per 100g of the various fractions were obtained.

Ferric Reducing Power Assay: [13] 0.5 ml of different concentrations (25-150 mcg/ml) of standard and samples were mixed with 0.5 ml of 0.2 M phosphate buffer (pH 6.6) and 0.5 ml of a 1% potassium ferricyanide solution. The mixture was incubated in a water bath at 50°C for 20 min. Subsequently, 0.5 ml of 10% (w/v) trichloroacetic acid solution was added, and the mixture was then centrifuged at 3000 rpm for 10mins. Finally, 0.5 ml of the supernatant layer solution was mixed with 0.5 ml of distilled water and 0.1 ml of 0.1% ferric chloride and the absorbance of the reaction mixture were measured at 700 nm. The experiment was performed in triplicate. The blank used here was

ethanol. A control was also prepared omitting the sample.

Hydrogen Peroxide Scavenging Assay: [11] 2.0 ml of the various concentrations of the standard and samples were mixed with 2.0 ml of 100mM hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. All samples were assayed in triplicates. Control used was all the reactants except the standard or sample.

Statistical analysis: Data were expressed as mean±SD. Statistical analysis were performed by using one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons of unpaired data. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION:

Estimation of Total Phenolic Content: The total phenolic content in the various fractions were found out by Folin Cio-calteau method. The absorbance values obtained for different concentrations of standard gallic acid are tabulated and the standard graph is shown in **Fig.1**.

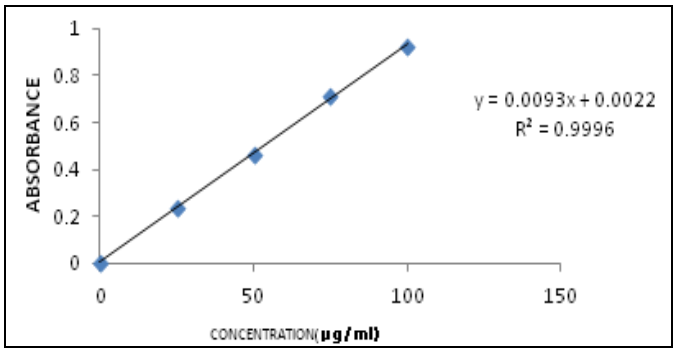

FIG.1: STANDARD GRAPH OF GALLIC ACID

The absorbance values for the various extracts were recorded and the total phenolic content in gallic acid equivalent are given in the **Table 1**.

TABLE 1: ESTIMATION OF TOTAL PHENOL CONTENTS OF THE VARIOUS FRACTIONS

Extracts	Gram equivalent of gallic acid per 100g
GH	3.88
GC	5.76
GE	13.74
GAL	4.62

The result shows that the ethyl acetate fraction (GE) has the highest gram equivalent of gallic acid per 100g. Thus the total phenolic content was found high for the ethyl acetate fraction.

Estimation of Total Flavonoid Content: Estimation of total flavonoids in the various fractions were carried out by using aluminium chloride colorimetric method. The absorbance values obtained for different concentrations of the standard rutin are tabulated and the standard graph in **Fig. 2**

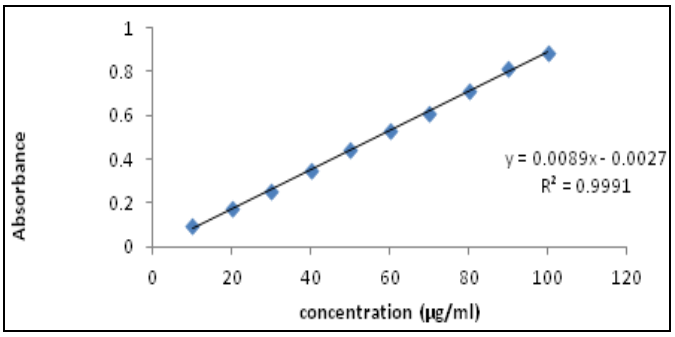

FIG. 2: STANDARD GRAPH OF RUTIN

The absorbance values for the various extracts are recorded and the total flavonoid content as rutin equivalent per 100 g are given in the table no:2.

TABLE 2: ESTIMATION OF TOTAL FLAVONOID CONTENT OF THE VARIOUS EXTRACTS

Extracts	Gram equivalent of rutin per 100g
GH	1.592
GC	1.749
GE	3.816
GAL	4.929

The result shows that the alcoholic fraction (GAL) has the highest gram equivalent of rutin per 100g. Thus the total flavanoid content was found high in the alcoholic fraction.

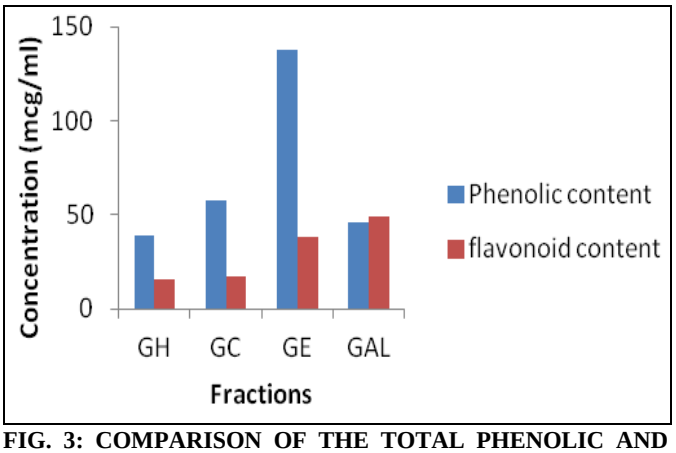

FIG. 3: COMPARISON OF THE TOTAL PHENOLIC AND FLAVONOID CONTENTS OF THE VARIOUS FRACTIONS

The leaves of *Garcinia brasiliensis* are both rich in phenolics and flavonoids.

Antioxidant Studies:

Ferric reducing power assay: The percentage inhibition obtained for the different concentrations of sample fractions were compared with the percentage inhibition obtained for the standard.

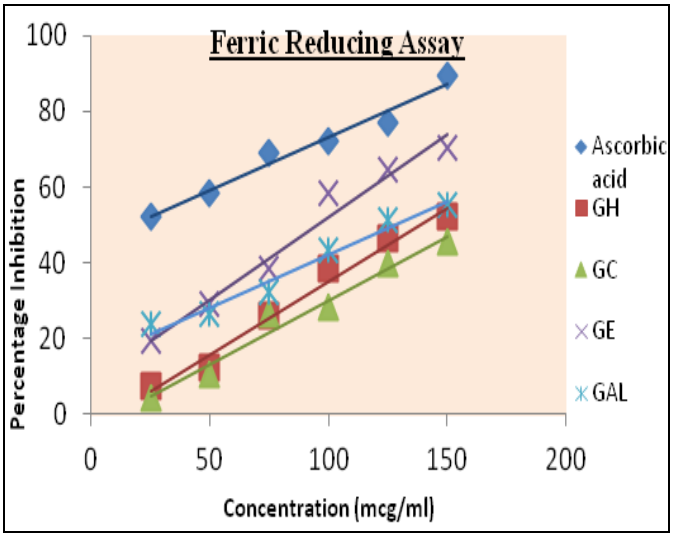

FIG. 4: COMPARISON OF THE PERCENTAGE INHIBITION OF THE VARIOUS FRACTIONS WITH THE STANDARD

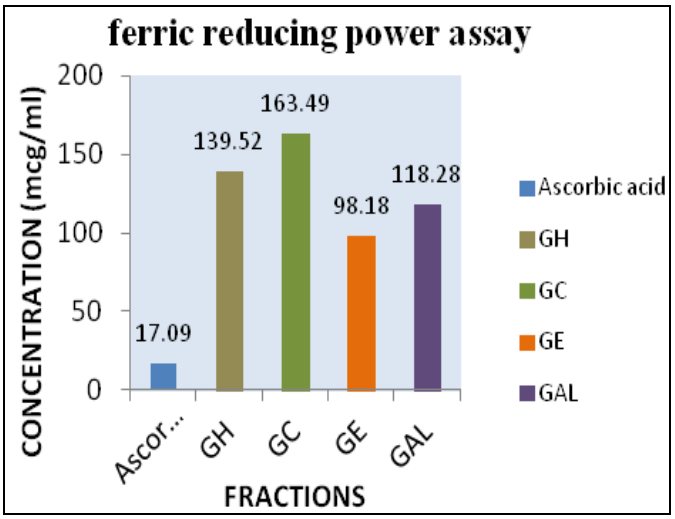

FIG. 5: COMPARISON OF THE IC_{50} VALUES OF THE VARIOUS FRACTIONS WITH THE STANDARD ASCORBIC ACID USING FERRIC REDUCING ASSAY

The ethylacetate fraction has an IC_{50} value of 98.18mcg/ml which is comparable with the IC_{50} value of the standard ascorbic acid (17.09mcg/ml) for the ferric reducing power assay.

Hydrogen peroxide scavenging assay: The percentage inhibition obtained for the different concentrations of sample fractions were compared with the percentage inhibition obtained for the standard.

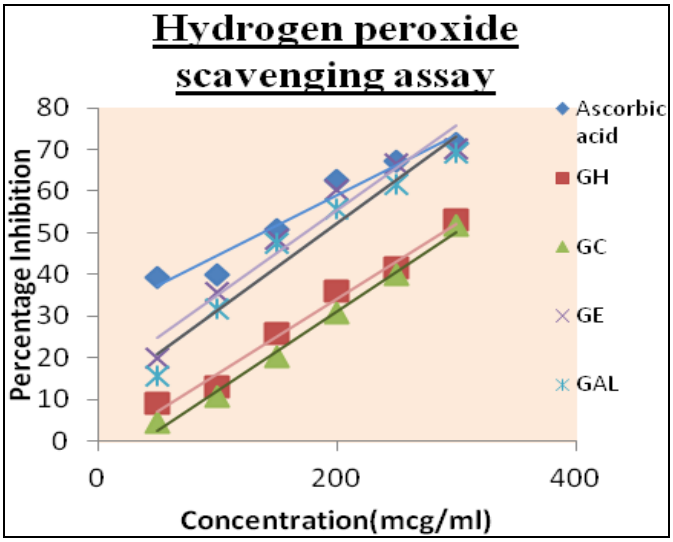

FIG. 6: COMPARISON OF THE PERCENTAGE INHIBITION OF THE VARIOUS FRACTIONS WITH THE STANDARD ASCORBIC ACID USING HYDROGEN PEROXIDE SCAVENGING

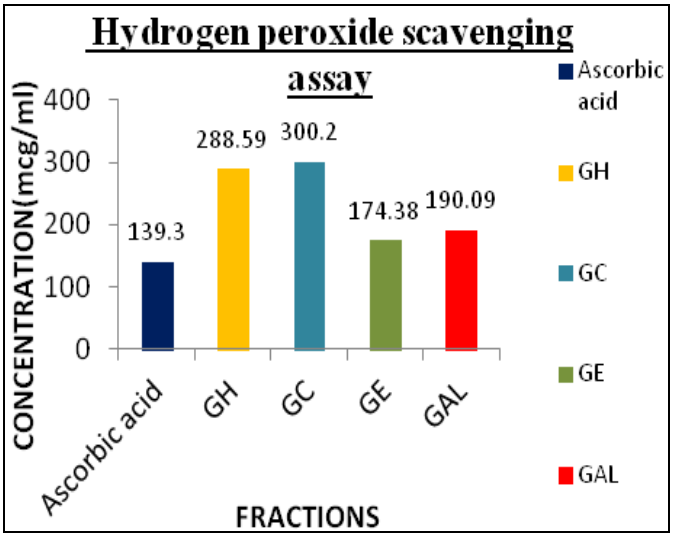

FIG. 5: COMPARISON OF THE IC_{50} VALUES OF THE VARIOUS FRACTIONS WITH THE STANDARD ASCORBIC ACID USING HYDROGEN PEROXIDE SCAVENGING ASSAY

The ethylacetate fraction has an IC_{50} value of 174.38mcg/ml which is comparable with the IC_{50} value of the standard ascorbic acid (139.3mcg/ml) for the hydrogen peroxide scavenging assay.

Statistical analysis is tabulated as below: **Table 4** Statistical data for hydrogen peroxide scavenging assay by the various fractions.

CONCLUSION: The estimation of total phenolics and flavonoids indicated the Garcinia brasiliensis to be rich in both phenolics and flavonoids Phenolic content of the ethylacetate fraction was found to be four times more than in the alcoholic fraction.

In alcoholic fraction, the phenolic content and the equal. Most of the activities by the plant extracts are attributed by the phenolics present.

The total phenolic content was 13.74g gallic acid equivalent per 100g for the ethylacetate fraction. This value was followed by the alcoholic fraction which showed a total phenolic content of 4.6g gallic acid equivalent per 100. The chloroform fraction and the n-hexane fraction had total phenolic content of 5.766g GAE per 100g and 3.88 GEA per 100g respectively. Total flavonoid content was expressed as rutin equivalents per 100g. The total flavonoid content showed by alcoholic fraction is 4.92g rutin equivalent per 100g followed by ethylacetate fraction, chloroform fraction and n-hexane fraction as 3.81g/100g, 1.74g/100g and 1.29g/100g respectively.

Ethylacetate fraction has three times more phenolic content than total flavonoid content whereas alcoholic fraction has almost same content of total phenolics and flavonoids.

The antioxidant activities of the various fractions were tested. The highest antioxidant activity was displayed by the ethylacetate fraction in scavenging hydroxyl free radical with an IC_{50} value of 174.38 mcg/ml. Ferric reducing power shown by the ethylacetate fraction (IC_{50}=98.13mcg/ml) was remarkable and was comparable with the standard ascorbic acid. The n-hexane fraction as well as the chloroform fraction showed significant antioxidant capacity when compared to the standard ascorbic acid in scavenging the hydrogen peroxide.

Thus the study found that the leaves of *Garcinia brasiliensis* have good antioxidant activity with highest antioxidant capacity demonstrated by the ethylacetate fraction. So this study has identified a new sustainable source of compounds with higher antioxidant activity.

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