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In-vitro assessment for antioxidant activity and phytochemical analysis of *Garcinia pedunculata*

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Abstract
In this study, the fruit of Garcinia pedunculata was screened for the presence of phytochemical compounds and antioxidant properties of the fruit in different extracts (aqueous, methanol, ethyl acetate and butanol, were compared and evaluated. Preliminary qualitative phytochemical screening of various extracts of the fruit confirmed the presence of alkaloids, phenols and tannins, flavonoids, saponins, proteins and carbohydrates. Phytochemical quantification of various extracts showed the highest total phenolic content with total flavonoid content of ***15.95 ± 0.29*** *mg QE/g,* ***21.39 ± 0.34*** *mg GAE/g in the aqueous extract of the plant. The same was also observed for TAC with aqueous showing the highest (****19.19 ± 0.36*** *mg AAE/g) and anti-oxidant activity with* IC_{50} *of* ***1.19*** *mg/ml for DPPH. Aqueous extract also showed the highest reducing capacity of* ***0.123 ± 0.0023*** *for FRAP.*

Keywords: Antioxidants, Garcinia pedunculata, phytochemicals, natural plant.

Introduction

A high dietary intake of fruits and vegetables is highly recommended as they possess several health benefits. Studies have reported that consumption of plant based diet significantly reduces the risk of developing stress related disorders due to the presence of antioxidants. Fruits and vegetables have been proven to have concentrations of vitamin C and A, electrolytes and phytochemicals, which are being identified as antioxidants(Arabshahi-Delouee and Urooj 2007). Major components of phytochemicals include alkaloids, glycosides, polyphenols (flavonoids and phenolic compounds) and terpenes. Diet rich in fruits and vegetables have also been proven to lower the chances of developing cancer (Ames 1983; Slavin and Lloyd 2012).

Since time immemorial, plants have been extensively used as traditional treatment and cure for various ailments. The knowledge of plants and their medicinal properties has led to an increase in the development of plant-based medicines that offer unique advantages like little to no side-effects, less toxic, cost effective and therapeutic effects (Scartezzini and Speroni 2000). According to WHO (World Health Organisation), 65% to 80% of the population of developing countries rely on traditional medicines for treatment of various ailments. WHO also encourages the safe and rational use of plants as traditional medicines for treatment (WHO 2011).

The use of natural products has witnessed a remarkable growth over the past years for their potential as a novel approach to medicinal therapy via phytochemicals for development of drugs (Chaachouay and Zidane 2024). Despite the advancement in the modern medicinal field, the use of medicinal plants continues to thrive due to their easy access, low-cost and having minimal to no side effect on patients(Veeresham 2012; Karimi *et al.* 2015). People living in rural areas and villages are highly dependent on plant therapy for treatment and survival, with little to no access to modern healthcare (Garcia 2020). About 25% of modern drugs and 60% of chemotherapeutic drugs available are chemical compounds derived from natural plant products and have been extensively used for medicinal treatment since the beginning of time (Gordaliza 2007; Newman and Cragg 2012). The unique properties of plants possessing phytochemicals make them the perfect choice of research areas such as their roles in inhibiting cancer cell progression (Hosseini and Ghorbani 2015). In recent times, traditional medicines have gained immense popularity and have been aggressively marketed as diet supplements (Ekor 2014).

Garcinia pedunculata Roxb. (**Fig. 1.**) which belongs to Kingdom: Plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Malpighiales; Family: Clusiaceae; Genus: *Garcinia*; Species: *pedunculata*. *Garcinia pedunculata* (GP) also known as BorThekera in Assam, Taikor in Bangladesh, Amlavetasa in India and Soh Danei in Meghalaya, is widely distributed throughout Northeast region of India (Sawian*et al.* 2007; Mundugaru*et al.* 2014; Islam *et al.* 2015; Bhuyan *et al.* 2020). Traditionally, this plant has been used for the treatment of various ailments. Local practitioners use the fruits for treatment of obesity, digestive disorders, gastric problems, detoxification for food poisoning and body cleanse due to the use of wrong

drugs (Sarma and Devi 2015). Methanolic and ethanolic extract of *Garcinia pedunculata* pericarp showed promising results as treatments for urinary tract infections (Zoliansanga and Lalfakzuala 2021). Methanolic extract of the fruit of *G.pedunculata* significantly decreased the levels of LDL-cholesterol and VLDL-cholesterol while increasing HDL cholesterol level in high fat induced rats, suggesting an efficient way to treat hyperlipidemia (Sarma *et al.* 2016). Other reported benefits of GP includes antibacterial, hepatoprotective, anti-inflammatory, cardioprotective, nephroprotective, antioxidant and anti-pyretic (Mundugaru*et al.*2016; Bhuyan *et al.* 2020; Basak *et al.* 2021, Zoliangsanga and Lalfakzuala 2021). Antimutagenic property of GP was first reported in the extracts of fruit rinds (Jayaprakasha *et al.*2006).

In the current study, we aim to determine the phytochemical compounds present in different solvent extracts and to also evaluate the antioxidant properties of *Garcinia pedunculata.*

Fig. 1. Fruit of *Garcinia pedunculata*.

Materials and methods

Plant material

Fresh fruits of *Garcinia pedunculata* were collected from Ri-Bhoi district of Meghalaya, India. The fruits were washed thoroughly with tap water and then distilled water. The fruits were peeled off, deseeded and cut into thin slices. The fruit slices were dried in an oven at less than 40 ºC for 5-7 days. The dried fruit slices were grounded using an electrical blender. The powdered form of the dried fruit was weighed and stored in an airtight bottle at 4 ºC for further analysis.

Extract preparation

The extracts were prepared by dissolving 10 g of powdered fruit material in 100 ml of solvent in a beaker, at the ratio of 1:10 (powder:solvent). The beaker was covered using an aluminium foil and the powder+solvent was subjected to magnetic stirring for 24 h. Themethanolic, ethyl acetate and butanolic filtrates were collected by filtering the extract through muslin cloth followed by Whatman # 1 filter paper. The filtrates were then concentrated by using a Rotary Evaporator and lyophilised to obtain the powdered form of the extract, whereas the aqueous extract was directly lyophilised. The lyophilised form of the extract was dissolved in normal saline solution at desired concentration and stored at 4℃ for further use.

Chemicals

Standards, such as DPPH, ascorbic acid, gallic acid and tannic acid were purchased from SRL. Folin-Ciocalteu's reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, potassium dihydrogen orthophosphate, sodium hydroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride and others chemicals used in the study were of analytical grade and purchased from Sigma-Aldrich and HiMedia laboratory, India.

Phytochemical Screening

Qualitative analysis

The lyophilised samples were screened for the presence of phytochemical constituents such as flavonoids (Zhishen*et al.* 1999; Edeoga*et al.* 2005); saponins (Kokate 1999); tannins (Trease and Evans 1989; Ainsworth and Gillespie, 2007);alkaloids (Harborne 1980; Evans 1997); polyphenols (Ainsworth and Gillespie 2007); anthraquinones (Trease and Evans 1996).

Quantitative analysis

- ***Estimation of total flavonoid content*** **(TFC)**

The amount of TFC present in the plant extracts was determined spectrophotometrically by the aluminium chloride method with slight modifications (Arvouet-grand *et al.* 1994). An aliquot of 1 ml sample (0.1-1 mg/ml) was added to 1 ml of 2% aluminium chloride (dissolved in methanol). The reaction mixture was allowed to stand at room temperature for 1 h after which the absorbance was read at 415 nm against reagent blank. Quercetin was used as a standard reference. The TFC was expressed as mg Quercetin Equivalent/g dried weight of extract and calculated by

the formula, TFC = (C x V)/M where, C is the concentration of quercetin (mg/ml), V is the volume of the extract in ml and M is the mass of the plant sample (g).

- ***Estimation of total phenolic content*** **(TPC)**

The amount of the TPCwas determined by the Folin-Ciocalteu method with slight modifications (Singleton*et al.* 1999). In brief, 1 ml of sample extract (0.1-1 mg/ml) was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted 1:10 with distilled water). This was allowed to stand for 5 min after which 4 ml of 7.5% sodium carbonate solution was added. The reaction mixture was incubated in the dark at room temperature for 2 h and the absorbance read at 740 nm against reagent blank. Gallic acid was used as a standard reference. The TPC was expressed as mg gallic acid equivalents per g dried sample and calculated by the formula , TPC = (C x V)/M, where C is the concentration of the gallic acid (mg/ml), V is the volume of the sample in ml and M is the mass of the sample extract in g.

- ***Estimation of total tannin content*** **(TTC)**

TTC was determined by a slightly modified Folin-Ciocalteu method (Ainsworth and Gillespie 2007).0.1 ml of sample extract was first dissolved in 7.5 ml distilled water followed by addition of Folin-Ciocalteu reagent (previously diluted 1:10 with distilled water). To this mixture, 1 ml of 35% sodium carbonate solution was added and the final volume of the solution was made to 10 ml. This was then incubated at room temperature for 30 min after which the absorbance was read at 700nm. Tannic acid was used as a reference standard. The TTC was expressed as mg tannic acid equivalent per g dried sample and calculated by the formula, TTC= (C x V)/M, where C is the concentration of tannic acid (mg/ml), V is the volume of sample extract(ml) and M is the mass of the sample (g).

In-vitro **Antioxidant Activity**

- ***DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay***

The ability of the plant extracts to scavenge the DPPH radical was determined *in-vitro* according to the method with slight modifications (Karadag *et al.* 2009). An aliquot of 1ml of plant extracts of varying concentrations (0.1-1 mg/ml) was added to 2 ml of DPPH reagent (0.004% dissolved in methanol). The reaction mixtures were incubated in the dark for 30 min after which the samples were read at 517 nm. A control was prepared without the sample. Ascorbic acid was used as the standard reference. The

scavenging activity of the samples was expressed as % inhibition and calculated according to the formula below:

Scavenging activity/ % inhibition = [(absorbance$_{control}$ -absorbance$_{sample)}$/absorbance$_{control}$] x 100

A decrease in absorbance signifies higher DPPH scavenging activity.

- ***Total reducing power assay***

This assay is based on the ability of plant extracts to reduce Fe^{3+} to Fe^{2+} and the total reducing power was obtained by using the Ferric Reducing Antioxidant Power (FRAP) method (Deore *et al.* 2009). An aliquot of 1 ml of sample of varying concentrations (0.1-1 mg/ml) was added to 1 ml of phosphate buffer (0.2 M, ph 6.6) and 1 ml of 1% potassium ferricyanide [($K_3Fe(CN)_6$]. The mixture was incubated at 50℃ for 30 min followed by the addition of 1 ml of 10% trichloroacetic acid (TCA). This was then centrifuged at 3000 rpm for 10 min. 1 ml of the supernatant was taken and mixed with 1 ml distilled water and 0.2 ml of 0.1% fresh ferric chloride ($FeCl_3.6H_2O$). After 5 min, the absorbance of the mixtures was measured at 700 nm against a blank containing all components except samples. Ascorbic acid was used as a standard reference. An increase in absorbance indicates a higher reducing power.

- ***Phosphomolybdenum assay***

The total antioxidant capacity (TAC) of the plant extracts was determined using the phosphomolybdenum method with slight modifications (Prieto *et al.* 1999). An aliquot of 0.1 ml of sample of varying concentrations (0.1–1 mg/ml) was mixed with 1 ml of reagent solution containing 28mM sodium phosphate, 4mM ammonium molybdate and 0.6 M sulphuric acid. The mixture was incubated at 95℃ for 90 min. The mixture was allowed to cool down to room temperature after which the absorbance was read at 695 nm against a blank containing solvent and methanol. Ascorbic acid was used as a standard reference. A control was prepared by adding all the reagents except samples. The TAC was expressed in mg ascorbic acid equivalent/g dry weight of extract and calculated by the formula TAC = (CxV)/M where C is the concentration of the standard ascorbic acid, V is the volume of the plant extract in ml, M is the mass of the plant extract.

Statistical analysis

All experiments were done in triplicates and the results were expressed as mean values ±SEM. Linear regression analysis was used to calculate IC_{50} for each plant extract.

Results

Qualitative analyses

Preliminary phytochemicals screening revealed the presence of major classes of phytochemical compounds in the different extracts of the plant sample (**Table 1**). The phytochemical screening was performed with aqueous, methanol, butanol and ethyl acetate extracts of *Garcinia pedunculata.*

Table 1: Qualitative analyses of phytochemical substances in different extracts of *Garcinia pedunculata.*

Compound	Aqueous	Methanol	Butanol	Ethyl acetate
Alkaloids	+	+	-	+
Flavonoids	++	+	+	+
Phenolic compounds	++	+	+	+
Tannins	+	+	+	+
Saponins	++	+	-	+
Proteins	+	+	+	+
Carbohydrates	+	+	+	+

++: highly present, +: present, -: absent

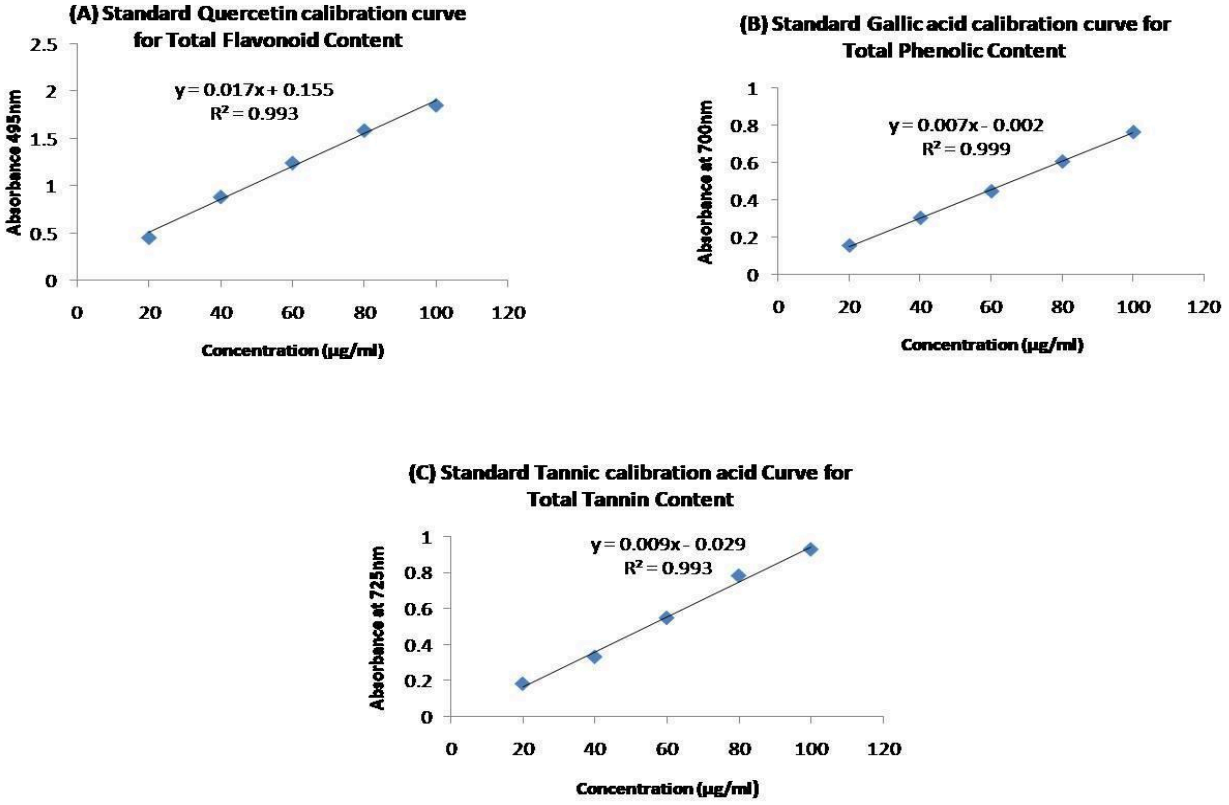

Fig. 2. Calibrative curve for Quercetin, Gallic acid and Tannic acid for TFC (A), TPC (B) and TTC (C) respectively.

Quantitative analyses

The TFC, TPC and TTC content in all extracts were calculated using the linear equation obtained from the standard curve as shown in figure 1, where y is the absorbance and x is the amount of quercetin equivalent (QE), gallic acid equivalent (GAE) and tannic acid equivalent (TAE) for TFC, TPC and TTC respectively. The TFC (21.39 ± 0.34),TPC (15.95 ± 0.29) and TTC (9.66 ± 0.10) was found to be highest in aqueous extract with butanol extract showing the lowest TFC (0.65 ± 0.02), TPC (6.34 ± 0.33) and TTC (2.66 ± 0.10), as observed in table 2. The TFC, TPC and TTC of the four extracts were found in decreasing order of aqueous> ethyl acetate> methanol > butanol for all the analyses.

***In-vitro* Antioxidant Activity**

- ***DPPH assay*** Ascorbic acid, widely used antioxidant standard, was used as a positive control and the calculated % inhibition values of both standards and samples was plotted against their respective concentrations as shown in **Fig. 3**. The results were

expressed as IC_{50} which is the concentration of standard/sample required to scavenge 50% of the DPPH radical. Low IC_{50} value indicates the highest scavenging activity. The aqueous extract showed the highest antioxidant activity as it had the lowest IC_{50} when compared to the other solvents. **Table 3** shows the scavenging effect of samples on DPPH radical and were in the following order: aqueous>methanol> butanol> ethyl acetate.

Table 2: Total flavonoid content, total phenolic content and total tannic content (equivalent/mg dry weight of extract)of different extracts of *Garcinia pedunculata*. Results expressed as mg gallic acid equivalents/g dry weight, mg quercetin equivalents/ g dry weight and mg tannic acid.

Extracts	Concentration (mg/ml)	Total Flavonoid Content (mg GAE/g)	Total Phenolic Content (mg QE/g)	Total Tannic Content (mg TAE/g)
Aqueous	1	21.39 ± 0.34	15.95 ± 0.29	9.66 ± 0.10
Methanol	1	3.71 ± 0.14	9.29 ± 0.22	5.08 ± 0.16
Butanol	1	0.65 ± 0.02	6.34 ± 0.33	2.66 ± 0.10
Ethyl acetate	1	17.49 ± 0.32	12.10 ± 0.29	6.99 ± 0.13

Data represented as Mean ± SEM, where n=3.

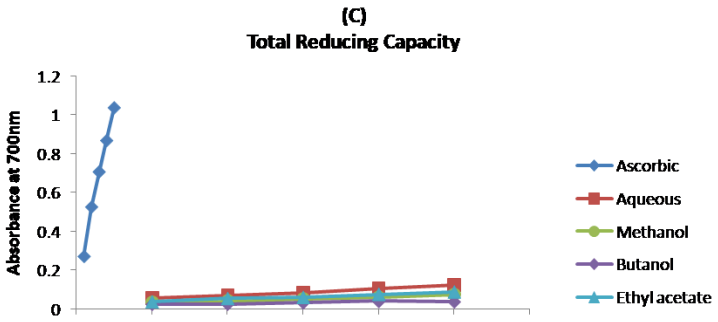

Fig. 3. Percentage inhibition of standard and various solvent extracts on DPPH (A), Absorbance of Total Antioxidant capacity of different extracts (B) and Absorbance of Total Reducing Capacity/FRAP (C), of *Garcinia pedunculata*.***Phosphomolybdenum assay (Total antioxidant Capacity).***

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents (AAE). **Fig. 4.** shows the absorbance reading of various extracts of the fruit against standard ascorbic acid. The TAC of various solvent samples was found to decrease in this order: aqueous> methanol> ethyl acetate> butanol as shown in **Table 4**. All results showed activity in a dose dependent manner.

Table 3: DPPH percentage inhibition of ascorbic acid and different solvent extracts.

	Ascorbic acid	Aqueous	Methanol	Butanol	Ethyl acetate
Concentration (mg/ml)	0.1	1	1	1	1
% inhibition (Mean±SEM)	52.61 ± 0.65	44.61 ± 0.12	39.51 ± 0.67	27.06 ± 0.21	25.24 ± 0.25
IC50 (mg/ml)	0.095	1.19	1.61	3.10	3.26

- ***Total Reducing Power Assay***

Absorbance reading of the various extracts shows a close comparison (**Fig. 5.**). Ascorbic acid being the standard displayed the strongest reducing power but when compared among the solvents it is evident that aqueous extract showed the highest and the other solvents decreased in this order: aqueous>ethyl acetate> methanol> butanol (**Table 5**).

Table 4: Total antioxidant content of different solvent extracts of *Garcinia pedunculata.* Data represented as Mean ± SEM, where n=3.

	Aqueous	Methanol	Butanol	Ethyl acetate
Concentration (mg/ml)	1	1	1	1
TAC (mg AAE/G)	19.19 ± 0.36	12.42 ± 0.43	6.11 ± 0.73	9.44 ± 0.17

Table 5: Results of total reducing power of different solvent extracts of *Garcinia pedunculata.*

	Ascorbic acid	Aqueous	Methanol	Butanol	Ethyl acetate
Concentration (mg/ml)	0.1	1	1	1	1
Absorbance (Mean±SEM)	1.047 ± 0.0043	0.123 ± 0.0023	0.073 ± 0.0012	0.038 ± 0.0008	0.084 ± 0.0018

Discussion

The use of plants to treat sickness and diseases has been in existence since old age. This is because medicinal plants possess biologically active compounds such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, etc. these compounds have been proven to possess activities such as antioxidant, anti-inflammation, anti-bacterial, anticancer, etc, each through different mechanisms (Bharti *et al*. 2012;Greenwell and Rahman 2015; Shingala *et al*. 2021; Wasihun *et al.* 2023).

Isolation of these biologically active compounds from their crude form is highly dependent on the type of solvent they are most soluble and displaying antioxidant activities through the different techniques. In this study, we have observed that aqueous extract showed the highest antioxidant activity.Phytochemical screening showed all compounds such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, proteins and carbohydrates to be present in the different solvents with the exception of butanol showing no presence of alkaloids and saponins. Quantitatively, aqueous extracts showed the highest yield for TFC (21.39 ± 0.34),TPC (15.95 ± 0.29) and TTC (9.66 ± 0.10) with butanol showing the lowest. The descending order of TFC, TPC and TTC for all solvent extracts are in the order of aqueous>ethyl acetate>methanol>butanol.

The ability of the solvent extracts to quench DPPH free radicals, converting the purple-colored DPPH solution to colorless product 2,2-diphenyl-1-picryl hydrazine displays the scavenging effects of the plant sample; the solvent extracts showed significant scavenging effects on the DPPH radical which was increasing with increase in the concentration of the extracts. In this study, we observed that the aqueous extract showed the highest DPPH scavenging activity amongst the four

solvents chosen and were found to decrease in the order aqueous>methanol>butanol>ethyl acetate. The IC_{50} calculated also showed the anti-oxidant activity of aqueous extract to be the lowest, with the value of 1.19.

A similar trend is also observed when assayed for total antioxidant activity which measures the scavenging ability of the antioxidants in the extract for free radicals with aqueous extract demonstrating the highest antioxidant capacity for phosphomolybdate reaction and were found to decrease in the order of aqueous>methanol>ethyl acetate>butanol. Studies have shown that many flavonoids and polyphenols have contributed to the total antioxidant activity of medicinal plants (Pietta 2000; Pandey and Rizvi 2009).

The reducing power of the different solvent extracts of the plant was assayed and it was observed that higher the absorbance, the stronger is the antioxidant activity; thus, the reducing power of the extracts also increases with the increase in concentration. Amongst the solvent extracts, the aqueous extract showed the highest reducing power capacity while butanol showed the lowest. The reducing power capacity of the four solvents were in decreasing order of aqueous> ethyl acetate> methanol> butanol.

All assays supported the same trend as TFC,TPC and TTC which strongly suggests that aqueous solvent is an excellent choice for extraction, due to the presence of the biologically active phytochemicals, which are responsible for the antioxidant activity of *Garcinia pedunculata.*

Conclusion

The use of plants as herbal medicines has been proven to be highly effective and popular for treatment of several ailments as it is safe, low cost and with lesser side effects as compared to synthetic drugs.Herbal remedies and traditional medicines are popular worldwide, from as simple as adding ginger to tea aiding in digestion to novel anti-cancer research. They are being consumed by the majority population without even realizing it. The present study showed *Garcinia pedunculata* as a rich source of secondary metabolites/phytochemicals including flavonoids, phenolic compounds, tannins and alkaloids which contributed to the antioxidant activity exhibited by the plant. The study also compared different solvents for extraction with aqueous extract displaying the most prominent results as compared to methanol, butanol and ethyl

acetate.*Garcinia pedunculata* thus proved to be a plant of interest with a diverse application in biological and pharmacological activities.

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