

Preliminary phytochemical screening, *in vitro* antioxidant activity, total polyphenolic and flavonoid content of *Garcinia lanceifolia* Roxb. and *Citrus maxima* (Burm.) Merr.

Partha Sarathi Bairy, Nilutpal Sharma Bora*, Bibhuti Bhusan Kakoti, Aparoop Das, Lalit Mohan Nainwal, Barnali Gogoi

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

ARTICLE INFO

Article history:

Received on: 23/02/2016

Revised on: 14/03/2016

Accepted on: 29/06/2016

Available online: 26/09/2016

Key words:

Antioxidant, reactive oxygen species, *Garcinia lanceifolia*, *Citrus maxima*, flavonoids, phenolic compounds, Assam.

ABSTRACT

Garcinia lanceifolia and *Citrus maxima* are two indigenous fruits of East India, particularly Assam; which has been used in various folkloric medicines to treat disorders ranging from diarrhea and jaundice to stomach and heart problems. The main aim of this study was to establish and compare the antioxidant potential of these two plants. The methanolic extracts of the bark of *G. lanceifolia* and *C. maxima* were tested for their antioxidant potential using various established *in vitro* assay procedures. The estimation of the total phenolic and total flavonoid content were also carried out. The phytochemical screening was also done prior to these assays, and it revealed that both the extracts were found to contain tannins and phenolic compounds and flavonoids along with other phytoconstituents. The results reveal that both these plants have a considerable amount of antioxidant activity which can be compared with each other and also the standards.

INTRODUCTION

Free radicals or reactive oxygen species (ROS) are generated as a result of several biological reactions or from exogenous factors and include superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxides. These ROS have various physiological roles *in-vivo* like intercellular signaling, phagocytosis and energy production (Nandy *et al.*, 2012). However the overproduction of these species may lead to damaged proteins, lipids and DNA, which in turn are associated with diseases like coronary artery disease, hypertension, diabetes, cancer, etc. Most of these ROS are scavenged or inactivated by various endogenous systems such as superoxide dismutase, catalase and peroxidase-glutathione system. But these systems may not be efficient enough to meet up with the demands of the

body which leads to the dependence on exogenously available antioxidants (Sre *et al.*, 2012). There has been resurgence in the interest in antioxidants from natural sources in recent years due to the restriction on the use of synthetic antioxidants because of their possible unwanted side effects. Hence, development of antioxidants from plant species has attracted lots of attraction and is considered desirable for prevention of many diseases (Vishwanathan *et al.*, 2013). India is one of the 12 mega biodiversity countries and it is gifted with an enormous wealth of biodiversity. It has diverse climatic zones and wealth of vibrant ethnomedicinal traditions. There are about 45,000 plant species and 81,000 animal species recorded in India so far. Northeast Indian states are the store house of medicinal plants which are naturally grown and available in the forests. It is known for high ethnic and biological diversity and is often referred to as biological hotspot (Hajra *et al.*, 2011). *Garcinia lanceifolia* Roxb, belonging to the family (Clusiaceae) is an important and endemic medicinal plant found in Assam, India. It is commonly known as “Rupahi-thekera” (Assamese), “Pelh” (Mizo), “Rupohi tekera” (Mising).

* Corresponding Author

Nilutpal Sharma Bora, Department of Pharmaceutical Sciences,
Dibrugarh University, Dibrugarh, Assam, India.
mail id: nilutpalsharma3 @ gmail.com

The plant is a handsome, small, evergreen tree; glabrous and grows up to a height of 12 feet under the dense shade of other trees. The fruits are eaten raw or made into pickles. It has been used in folkloric medicine to treat stomach disorders, in worm infestations, diarrhea and jaundice (Bora *et al.*, 2014). *Citrus grandis* (L.) Osb. or *Citrus maxima* (Burm.) Merr., belonging to the family Rutaceae is an indigenous plant belonging to the tropical parts of Asia. It is commonly known as "Pomelo or Shaddock" (English), Robab Tenga (Assamese), Jambura (Bengali).

The plant flowers between April to June and is reported to be used as stomachic, cardiac stimulant, antioxidant and as an appetizer. It is also reported to be used in cases of anemia (Merina *et al.*, 2012; Mahbubur Rahman *et al.*, 2013). Both of these plants are freely available to the people residing in the tropical South-East Asian countries.

Hence, if proven scientifically; we can use these plants as a readily available and easily accessible, low cost source of natural antioxidants. Therefore an exhaustive effort was made to determine the antioxidant and free radical scavenging activity of the bark of the plants of *Garcinia lanceifolia* Roxb. and *Citrus maxima* (Burm.) Merr.

MATERIALS AND METHODS

Drugs and Chemicals

Chemicals like 2-Deoxy- D- Ribose, Ethylene Diamine Tetra Acetic Acid Disodium Salt, Nitro blue tetrazolium chloride (NBT), NADH (reduced) disodium salt were obtained from SRL Sisco Research Laboratories Pvt. Ltd. (Mumbai, India); 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) from Sigma-Aldrich Chemical Co. (Steinheim, Germany). Solvents like methanol, chloroform, petroleum ether, acetone, di-ethyl ether and all other chemicals were of analytical grade (AR) and were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India) and Himedia Laboratory, (Mumbai, India).

Instrumentation

All the spectrophotometric determinations were carried out using a double-beam spectrophotometer with split-beam technology, model SPECORD® 50 PLUS, make Analytik Jena AG, Germany.

Botanical material

The barks of *Garcinia lanceifolia* and *Citrus maxima* were collected during the month of August, 2013 from the campus of Dibrugarh University and neighboring areas of Dibrugarh, Assam, India.

The taxonomical identification was done by Dr. A. A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong vide identification number BSI/ERC/2014/Plant identification/882. The voucher specimen of the plant was deposited in the Research Lab of the Department for further references.

Preparation and selection of bark extracts

The bark was washed thoroughly with running tap water and cut into pieces. The bark was then dried partially under sunlight and partially under the shade for a week. The dried bark pieces were then ground in mechanical grinder and stored in airtight containers free from moisture. The dried crude drug was macerated for 48 hours in petroleum ether which defatted the drug and cleared the material of any deposited debris like mold, dirt etc. The drug was packed in a soxhlet extractor and extracted successively with solvents in increasing order of their polarity. The order of the solvents was petroleum ether<chloroform<ethyl acetate<methanol. The drugs were extracted using each solvent for 72 hours each or until the drug got exhausted of its phytochemical constituents. The extraction with solvents of different polarity facilitated all the constituents present in the drug to get extracted. The extracts were filtered using a muslin cloth to obtain a liquid extract which was further concentrated using a rotary evaporator at a temperature of $(40 \pm 5^\circ\text{C})$, model Rotavapor® R II, BUCHI, Switzerland. Preliminary phytochemical tests were carried out with all the extracts in order to evaluate for the presence of different phytochemical constituents. The most polar solvent in the whole process, i.e. only the methanolic extracts of both the plant barks had showed the presence of flavonoids and phenolic compounds. The methanolic extract of both the plants were selected for this study because crude extracts rich in phenolic compounds have been associated with the capability to retard oxidative degeneration (Kähkönen *et al.*, 1999).

Phytochemical screening of the plant extract

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods with little modifications (Evans, 2009; Harborne 1998).

DPPH radical scavenging activity

The radical scavenging activity of the methanolic bark extracts of *Garcinia lanceifolia* (MEGL) and *Citrus maxima* (MECM) against stable DPPH was determined spectrophotometrically using the method as described by Blois, 1958 with trivial modifications in the method (Blois, 1958). The absorption maximum of a stable DPPH radical in methanol was at 517nm. When DPPH reacts with an antioxidant, which can donate hydrogen, it gets reduced. 1ml of 0.3mM DPPH solution was added to 2ml of each different concentrations of standard solution and incubated at dark for 30mins at room temperature after it has been shaken vigorously. 1ml of 0.3 mM DPPH solution was added to 2ml of methanol and this solution was taken as control and allowed to incubate at dark for 30mins at room temperature. After 30 min, absorbance was measured at 517 nm taking methanol as blank using UV-Visible spectrophotometer. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate. Ascorbic acid was used as a reference compound. The

capability to scavenge the DPPH radical was calculated as the inhibition percentage of free radical by the sample/ standard using the following formula:

$$\% \text{ inhibition of DPPH scavenging activity} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A₀ is the absorbance of the control reaction and A_t is the absorbance of test/standard.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and MEGL/MECM for hydroxyl radical generated by Fe³⁺-Ascorbate-EDTA-H₂O₂ system (Fenton reaction) according to the method of Kunchandy and Rao with trivial modifications in the method (Kunchandy and Rao, 1990). Absorbance was measured spectrophotometrically at 532nm against control. To 0.5ml of each different concentrations of Quercetin (standard), sample extracts and water (control); 0.1 ml of 2-deoxy-2-ribose, 0.2ml of EDTA, 0.2 ml of ferric chloride and 0.1ml of H₂O₂ were added and incubated for 1hr at 37°C. After that 1ml of 1% TBA and 1ml of 2.8% TCA were added to the mixture and incubated at 100 °C for 20mins and then cooled and absorbance was taken at 532nm. Reactions were carried out in triplicate spectrophotometrically taking distilled water as blank. The percentage inhibition was determined against the standard by comparing the results of the test and control compounds. The hydroxyl radical scavenging activity of the extract was reported as % inhibition of deoxyribose degradation and was calculated as follows:

$$\% \text{ inhibition of Hydroxyl radical (OH } ^-\text{) scavenging activity} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A₀ is the absorbance of the control reaction and A_t is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extracts was expressed as IC₅₀ from the dose inhibition curve (Bhaumik *et al.*, 2008).

Nitric Oxide Radical Scavenging Activity

In this assay method, nitric oxide generated from sodium nitroprusside was measured by the Greiss Illosvoy reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Maccocci *et al.*, 1994) which interacts with oxygen to produce nitric ions that can be estimated by using Greiss reagent. Scavengers of nitric oxide compete with oxygen, which leads to reduced production of nitric oxide.

Absorbance was measured spectrophotometrically at 546nm against control. To 1ml of each of standard solution, sample extracts and control of different concentrations separately; 1ml of sodium nitroprusside and 1ml of phosphate buffer saline pH 7.4 were added and incubated at 25°C for 150 min. After

incubation, 1ml of the above mixture was taken and 1ml of Griess reagent was added to it and then allowed to stand in dark for 30mins and then absorbance was taken at 546nm. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was measured spectrophotometrically in triplicates and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess reagent. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample/ standard and was calculated using the following formula:

$$\% \text{ inhibition of nitric oxide radicals} = \frac{A_0 - A_t}{A_0} \times 100$$

Where, A₀ was the absorbance of the control, and A_t was the absorbance of test/ standard. The antioxidant activity of extracts was expressed as IC₅₀ value. The IC₅₀ value is defined as the concentration (µg / ml of dry extract that inhibits the formation of nitric oxide by 50%.

Hydrogen Peroxide Radical Scavenging Activity

The ability of the extracts to scavenge H₂O₂ was determined according to the method of Ruch *et al.* 1989. Absorbance was measured spectrophotometrically at 230nm. To 1ml of ascorbic acid solution and sample extracts of different concentrations, 0.6ml of H₂O₂ was added. Absorbance of H₂O₂ at 230 nm was determined after 10 min against a blank solution containing the phosphate buffer without H₂O₂. 1ml of Phosphate buffer and 0.6ml of H₂O₂ was used as a control. Reactions were carried out in triplicate spectrophotometrically. The percentage of H₂O₂ scavenging of both the extracts and standard compounds was calculated.

The percentage inhibition was calculated as:

$$\% \text{ inhibition of hydrogen peroxide radicals} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A₀ is the absorbance of the control reaction and A_t is the absorbance in the presence of the extracts. The antioxidant activity of the extract was expressed as IC₅₀. The IC₅₀ value is defined as the concentration (µg / ml) of dry extract that inhibits the formation of H₂O₂ radicals by 50%.

Determination of Reducing Power

The reducing power of test sample was determined on the basis of the ability of their antioxidant principles to form color complexes with potassium ferricyanide, TCA and FeCl₃ due to transformation of Fe³⁺ - Fe²⁺. The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity (Barreira *et al.*, 2008). Reducing the power of a methanolic bark extract of *Garcinia lanceifolia* (MEGL) and *Citrus maxima* (MECM) was determined according to the method of Oyaizu in 1986 with trivial modifications in the

method (Oyaizu, 1986). From each different concentrations of ascorbic acid, samples extracts and control (water), 1ml was taken and added to 2.5ml of phosphate buffer and 2.5ml of potassium ferricyanide. After that it was incubated at 50°C for 20mins. 20mins later 2.5ml of Trichloroacetic acid was added, centrifuged at 3000rpm for 10mins. 2.5ml of supernatant layer was taken, and then mixed with 2.5ml of distilled water and 0.5ml of ferric chloride. Absorbance of the resultant mixture was measured at 700nm spectrophotometrically. All the tests were performed in triplicate using distilled water as blank. Gradual enhancement of absorbance with concentration indicates the higher reducing power (Deore *et al.*, 2009).

Estimation of Total Phenolic Content

Total soluble phenolics in the extracts were determined according to the method used by Macdonald *et al.*, with trivial modifications using Gallic acid as a standard phenolic compound (Macdonald *et al.*, 2008). The phenol reacts with Phosphomolybdic acid in the presence of alkaline medium to produce blue color complex known as molybdenum blue complex. 1ml of each concentration of Gallic acid was mixed with 5ml of Folin-Ciocalteu reagent (diluted 10 fold) and 4ml of 0.7 M sodium carbonate. Absorbance was measured spectrophotometrically in triplicates at 765nm and calibration curve was plotted. 1ml of each concentration of extract solution was mixed with 5ml of Folin-Ciocalteu reagent (diluted 10 fold) and 4ml of 0.7 M sodium carbonate.

Absorbance was measured spectrophotometrically in triplicates at 765nm. 1ml of distilled water was mixed with 5ml of Folin-Ciocalteu reagent and 4ml of sodium carbonate. This was taken as a control and absorbance was measured spectrophotometrically at 765nm. The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

$$T = C \times \frac{V}{M}$$

where, T= Total phenolic contents, mg g⁻¹ plant extract, in Gallic Acid

Equivalent (GAE),

C= Concentration (mg ml⁻¹) of Gallic acid obtained from the calibration curve,

V= Volume of extract (ml),

M= Weight (mg) of methanolic plant extract.

Estimation of Total Flavonoid Content

Total soluble flavonoid content of the extracts was determined with aluminium chloride using quercetin as the standard according to the method of Ebrahimzaded *et al.*, with trivial modifications in the method (of Ebrahimzaded *et al.*, 2001). To 1ml of each different concentration of quercetin, 2ml of methanol was added to each concentration. Then it was mixed with 0.2ml of aluminium chloride and 0.2ml of potassium acetate and finally 5.6ml of distilled water was added to each concentration. To 1ml of of extract, 2ml of methanol was added.

Then it was mixed with 0.2ml of aluminium chloride and 0.2ml of potassium acetate and finally 5.6ml of distilled water was added to each concentration. All the samples were incubated for 30mins at room temperature and absorbance was measured at 415nm against control. The total flavonoid content in the fractions was determined as µg Quercetin equivalent by using the standard quercetin graph and using the following formula;

$$T = C \times \frac{V}{M}$$

where, T= Total flavonoid content, mg g⁻¹ plant extract, in Quercetin Equivalent (QE),

C= Concentration (mg ml⁻¹) of Quercetin obtained from the calibration curve,

V= Volume of extract (ml),

M= Weight (mg) of methanolic plant extract.

Statistical analysis

All data on measurement of antioxidant activities are the average of duplicate analyses. The raw analyses were performed in triplicate. The data were recorded as mean ± standard error of mean and analyzed by GraphPad PRISM (Version 5.03 for Windows December 10, 2009). One way analysis of variance was performed. Significant differences between means were determined by Dunnett's multiple comparison tests. Values of p < 0.05 were regarded as significant and p < 0.01 very significant.

RESULTS AND DISCUSSIONS

Phytochemical screening of the plant extracts

The qualitative phytochemical estimation of both the extracts was done and the results have been tabulated in Table 1. Both the extracts showed the presence of a number of important phytoconstituents out of which the tannins, phenolics and flavonoids were common in both and of the utmost importance. The MEGL also showed the presence of amino acids, carbohydrates, glycosides, lignins, proteins and triterpenoids. While the MECM showed the presence of the carbohydrates, flavonoids, glycosides and triterpenoids.

Table 1: Phytochemical screening of *G. lanceifolia* and *C. maxima* methanolic bark extracts.

Plant Constituents	Methanolic extract of the bark of <i>G. lanceifolia</i>	Methanolic extract of the bark of <i>C. maxima</i>
Alkaloids	-	-
Amino acids	+	-
Carbohydrates	+	+
Fats and Oils	-	-
Flavonoids	+	+
Glycosides	+	+
Gums	-	-
Lignins	+	-
Proteins	+	-
Steroids	-	-
Triterpenoids	+	+
Saponins	-	-
Tannins and Phenolic Compounds	+	+

“+” indicates the presence of the particular phytoconstituent.

“-” indicates the absence of the particular phytoconstituent.

DPPH radical scavenging activity

The scavenging ability of MEGL and MECM was compared with the standard ascorbic acid and is shown in Figure 1. The DPPH Scavenging activity of the two extracts and the standard was expressed as percentage inhibition. The MEGL showed a better scavenging effect on DPPH radicals than MECM. However, it was not as effective as the standard ascorbic acid. The scavenging activity of both the extracts was found to increase in a dose dependent manner. The MEGL showed antioxidant activity with IC_{50} value of 29.21 $\mu\text{g}/\text{ml}$ while MECM exhibited an IC_{50} value of 33.05 $\mu\text{g}/\text{ml}$. However the known antioxidant ascorbic acid exhibited an IC_{50} value of 17.37 $\mu\text{g}/\text{ml}$. At the concentration of 80 $\mu\text{g}/\text{mL}$, MEGL showed 81.33% inhibition while that of ascorbic acid was 87.41%. At other concentrations, percentage inhibition of MEGL was found to be marginally less than that of ascorbic acid.

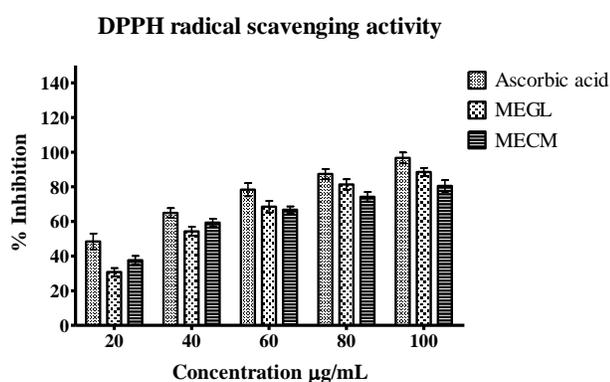


Fig. 1: Comparison of the DPPH radical scavenging activity of ascorbic acid (standard), methanolic extract of *Garcinia lanceifolia* bark (MEGL) and *Citrus maxima* bark (MECM), all values is expressed as Mean \pm S.E.M.

Hydroxyl Radical (OH⁻) Scavenging Activity

The scavenging ability of MEGL and MECM on OH⁻ radical is shown in Figure 2 and compared with ascorbic acid. The hydroxyl radical scavenging activity is apparent from the gradual increase in percentage scavenging activity with increasing concentration of methanolic bark extract of *Garcinia lanceifolia* and reference standard ascorbic acid. The IC_{50} values were found to be 291.85 $\mu\text{g}/\text{ml}$ for MEGL, 278.50 $\mu\text{g}/\text{ml}$ for MECM and 178.40 $\mu\text{g}/\text{ml}$ for ascorbic acid respectively.

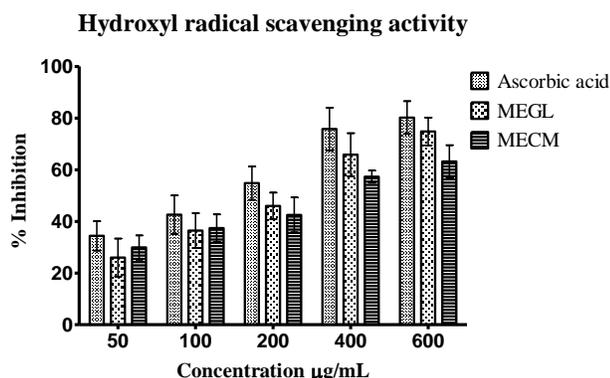


Fig. 2: Comparison of the hydroxyl radical scavenging activity of ascorbic acid (standard), methanolic extract of *Garcinia lanceifolia* bark (MEGL) and *Citrus maxima* bark (MECM), all values are expressed as Mean \pm S.E.M.

Nitric Oxide Radical Scavenging Activity

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by MEGL and MECM. *Garcinia lanceifolia* and *Citrus maxima* at varying concentrations showed a remarkable inhibitory effect of nitric oxide radical- scavenging activity (Figure 3). Results showed the percentage of inhibition in a dose dependent manner. The concentration of *Garcinia lanceifolia* needed for 50% inhibition (IC_{50}) was found to be 210.50 $\mu\text{g}/\text{ml}$, for *Citrus maxima* it was 338.2 $\mu\text{g}/\text{ml}$ whereas 89.03 $\mu\text{g}/\text{ml}$ was needed for curcumin respectively.

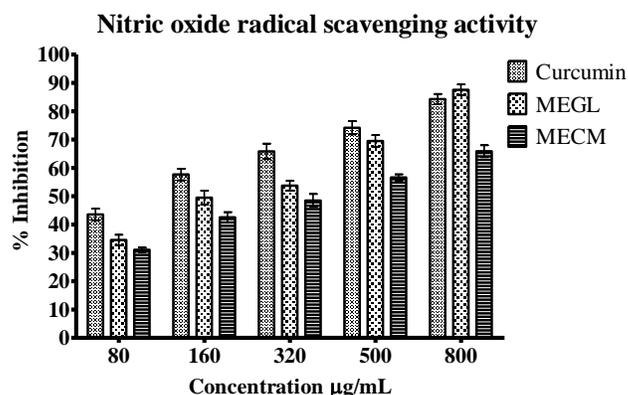


Fig. 3: Comparison of the nitric oxide radical scavenging activity of curcumin (standard), methanolic extract of *Garcinia lanceifolia* bark (MEGL) and *Citrus Maxima* bark (MECM), all values are expressed as Mean \pm S.E.M.

Hydrogen Peroxide Radical Scavenging Activity

Hydrogen peroxide scavenging activity of the extract is compared with ascorbic acid. The results are shown in Figure 4. The results showed that MEGL and MECM were effective in scavenging hydrogen peroxide in a dose dependant manner. The result is comparable with that of reference standard ascorbic acid. The IC_{50} value of ascorbic acid, MEGL and MECM are 117.13 $\mu\text{g}/\text{ml}$, 77.19 $\mu\text{g}/\text{ml}$ and 600 $\mu\text{g}/\text{ml}$ respectively. The results showed that MEGL was more effective in scavenging hydrogen peroxide than the standard. The result is comparable with that of reference standard ascorbic acid.

Hydrogen peroxide radical scavenging activity

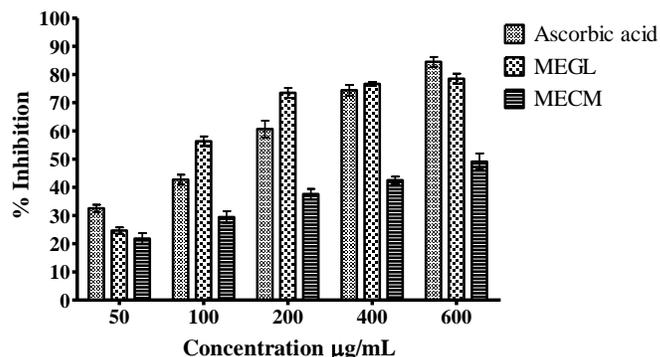


Fig. 4: Comparison of the hydrogen peroxide radical scavenging activity of ascorbic acid (standard), methanolic extract of *Garcinia lanceifolia* bark (MEGL) and *Citrus maxima* bark (MECM), all values are expressed as Mean \pm S.E.M.

Reducing power assay

The measurement of reducing capacity was for Fe^{3+} - Fe^{2+} transformation in the presence of the extract was estimated. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The results of reducing power of MEGL and MECM are shown in Figure 5. The result shows the extent of the reduction in terms of absorption value at 700nm. There is a direct association between antioxidant activity and reduction capacity. The reducing power was determined in comparison with standard drug Ascorbic acid and the extent of reduction exhibited in following order: ascorbic acid > MECM > MEGL. Reducing power will increase accordingly with the increase in absorbance.

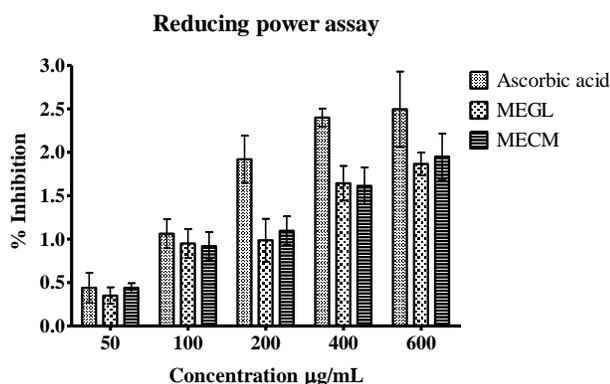


Fig. 5: Comparison of reducing power of ascorbic acid (standard), methanolic extract of *Garcinia lanceifolia* bark (MEGL) and *Citrus maxima* bark (MECM), all values are expressed as Mean \pm S.E.M.

Standard curve of gallic acid

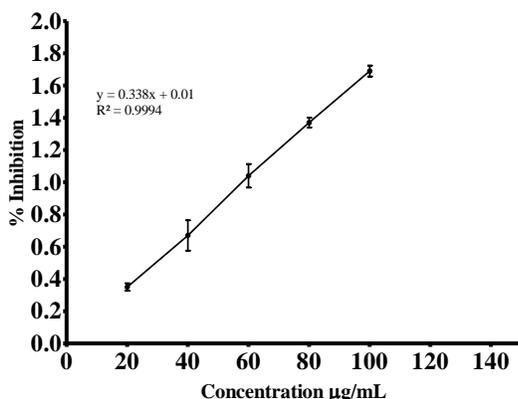


Fig. 6: Calibration curve of Gallic acid (standard), all values are expressed as Mean \pm S.E.M.

Estimation of Total Phenolic Content

The content of phenolic compound in the methanolic bark extract of *Garcinia lanceifolia* and *Citrus maxima* measured by Folin-Ciocalteu reagent in terms of Gallic acid equivalent (GAE) was 6.70 mg g^{-1} and 5.20 mg g^{-1} respectively. The amount of polyphenolic compounds present was derived from the absorbance of the sample solutions prepared and the standard which has been tabulated in Table 2. Figure 6 shows the calibration curve of Gallic acid. These results suggest that higher

levels of antioxidant activity were due to the presence of phenolic components.

Table 2: Observation of calibration curve in total phenolic estimation.

Concentration (mg ml^{-1})	Absorbance (Mean \pm S.E.M)
20	0.35 ± 0.023
40	0.67 ± 0.094
60	1.04 ± 0.073
80	1.37 ± 0.030
100	1.69 ± 1.034
MEGL	0.21 ± 0.022
MECM	0.15 ± 0.041

Estimation of total flavonoids

The content of flavonoid compound in the methanolic bark extract of *Garcinia lanceifolia* measured by aluminium chloride method in terms of Quercetin equivalent (QE) was found to be 61.17 mg g^{-1} and 48.54 mg g^{-1} respectively. The absorbance of the standard and the samples is tabulated in Table 3. Quercetin was used as the standard and the amount of the total flavonoids present were derived from it which is shown in Figure 7. It has been recognized that flavonoids exert considerable antioxidant activity and exhibit substantial beneficial effects on human nutrition and health.

Table 3: Observation of calibration curve in Total Flavonoid Estimation.

Concentration (mg ml^{-1})	Absorbance (Mean \pm S.E.M)
50	0.45 ± 0.01
100	0.85 ± 0.09
150	1.27 ± 0.26
200	1.67 ± 0.08
250	2.10 ± 0.34
MEGL	0.52 ± 0.08
MECM	0.33 ± 0.030

Standard curve of Quercetin

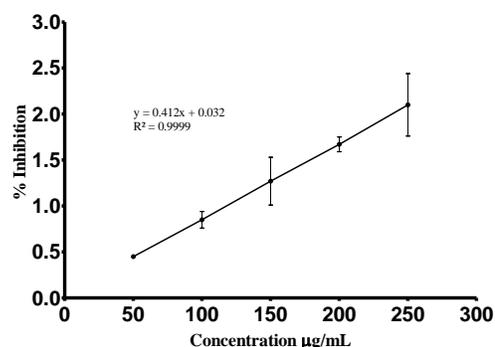


Fig. 6: Calibration curve of quercetin (standard), all values are expressed as Mean \pm S.E.M.

CONCLUSION

In conclusion from this study, it has been found that the methanolic extract of the bark of *Citrus maxima* and *Garcinia lanceifolia*, has a considerable amount of antioxidant activity which are comparable to the standard in some assay, while in some they are found to be more potent than that of the standard. This study provides us with a detailed overview of the antioxidant potential of two important, endemic and consumable plants of

Assam. These plants are consumed by the local people and since they are a source of very potent antioxidants they are providing us with answers to our various diseases and disorders. They are also a potential alternative to chemical antioxidants as they will be naturally available, cheaper and with fewer side effects.

ABBREVIATIONS

MEGL: Methanolic extract of the bark of *Garcinia lanceifolia*.

MECM: Methanolic extract of the bark of *Citrus maxima*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENTS

The authors are thankful to Department of Pharmaceutical Sciences, Dibrugarh University, India for providing the research facilities for this work.

Financial support and sponsorship: Authors are thankful to AICTE (All India Council of Technical Education), Research Promotion Scheme (RPS) 2011 for providing grant (Grant No: 8023/RIB/RPS-10/ (NER) /2011-12).

Conflict of interest: There are no conflicts of interest.

REFERENCES

- Barreira JC, Ferreira IC, Oliveira MB, Pereira JA. Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. *Food and Chemical Toxicology*. 2008;46(6):2230-5.
- Bhaumik UK, Kumar AD, Selvan VT, Saha P, Gupta M, Mazumder UK. Antioxidant and free radical scavenging property of methanol extract of *Blumea lanceolaria* leaf in different *in vitro* models. *Pharmacologyonline*. 2008;2:74-89.
- Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*, 1958; 181: 1199-1200.
- Bora NS, Kakoti BB, Gogoi BA. Investigation of *in vitro* anthelmintic activity of *Garcinia lanceifolia* bark in *Pheretima posthuma* (Indian adult earthworm). *PHARMANEST*. 2014;5(3):2007-10.
- Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology*. 2008;7(18).
- Evans WC. 2009. *Trease and Evans Pharmacognosy*. London: Saunders Limited.
- Halliwell B, Gutteridge JM. *Free radicals in biology and medicine*. Oxford University Press, USA; 2015 Jul 1.
- Harborne JB. 1998. *Phytochemical methods: A guide to modern techniques of plant analysis*. London: Chapman & Hall.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry*. 1999; 47(10):3954-62.

Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. *International Journal of Pharmacognosy*, 1990; 58(3): 237-240.

Marcocci L, Maguire JJ, Droylefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochemical and biophysical research communications*. 1994; 201(2): 748-55.

McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food chemistry*. 2001 Apr 30;73(1):73-84.

Merina N, Chandra KJ, Jibon K. Medicinal plants with potential anticancer activities: A Review. *Int Res J Pharm*. 2012;3(6):26-30.

Muruhan S, Selvaraj S, Viswanathan PK. *In vitro* antioxidant activities of Solanum surattense leaf extract. *Asian Pacific journal of tropical biomedicine*. 2013; 3(1):28-34.

Nandy S, Paul HS, Barman NR, Chakraborty B. *In vitro* evaluation of antioxidant activity of *Leucas plukenetii* (Roth) Spreng. *Asian J Plant Sci Res*. 2012; 2: 254-62.

Nath M, Dutta BK, Hajra PK. Medicinal plants used in major diseases by Dimasa tribe of Barak Valley. *Assam University Journal of Science and Technology*. 2011; 7(1):18-26.

Oyaizu M. Studies on products of browning reaction--antioxidative activities of products of browning reaction prepared from glucosamine. *Eiyogaku zasshi= Japanese journal of nutrition*. 1986.

Rahman AH, Biswas MC, Islam AK, Zaman AT. Assessment of Traditional Medicinal Plants Used by Local People of Monirampur Thana under Jessore District of Bangladesh. *Wudpecker Journal of Medicinal Plants*. 2013; 2(6): 099-109.

Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*. 1989; 10(6): 1003-8.

SL D, Khadabadi SS, Baviskar BA, Khangenbam RA, Koli US, Daga NP, Gadbaill PA, Jain PA. *In vitro* antioxidant activity and phenolic content of *Croton caudatum*. *In vitro*. 2009; 1(2): 174-6.

Sre PR, Sheila T, Murugesan K. Phytochemical screening and "in-vitro" anti-oxidant activity of methanolic root extract of *Erythrina indica*. *Asian Pacific Journal of Tropical Biomedicine*. 2012; 2(3): S1696-700.

How to cite this article:

Bairy PS, Bora NS, Kakoti BB, Das A, Nainwal LM, Gogoi B. Preliminary phytochemical screening, *in vitro* antioxidant activity, total polyphenolic and flavonoid content of *Garcinia lanceifolia* Roxb. and *Citrus maxima* (Burm.) Merr. *J App Pharm Sci*, 2016; 6 (09): 133-139.