A COMPARATIVE STUDY OF ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES OF *AEGLE MARMELOS* FRUIT PULP AVAILABLE IN KOKRAJHAR AREA

Bidanshree Basumatary and Monmi Saikia*
Department of Chemistry, Bodoland University, Kokrajhar, BTAD, Assam
*For correspondence. (monmi.saikia@gmail.com)

Abstract: *Aegle marmelos* fruit pulp is medicinally important, cures diabetes and different part of it has been used generally as a remedy for gastrointestinal infections, anaemia, fractures, asthama, healing of swollen joints, controlling blood pressure, jundice as well as many more problems of human. In this study we want to reveal a comparative study of the antioxidant potentials of Aegle marmelos fruit pulp extracts in different solvents available in Kokrajhar area. Standard methods were adopted to screen antioxidant and phytochemical nature of A. marmelos fruit pulp and results are compared of.

Keywords: Aegle marmelos; pulp; antioxidant property; phytochemicals; medicinal value

1. Introduction:

India is a treasure trove of aromatic and medicinal plants. Today medicinal plants play a major role as pillar of traditional healthcare systems of medicine in many developing countries. Since from the ancient times, different drugs have been formulated using the bioactive compounds present in these medicinal plants. More than 60% of the world's population depends on phytomedicines derived from these medicinal plants for primary health care needs. The Phytoconstituents from these medicinal plants serve as lead compounds in the modern era in drug discovery and design. The most important phytoconstituents which are biologically active in plants are Tannins, Flavonoids, Alkaloids and Phenolic compounds [1]. *Aegle marmelos* belongs to Rutaceae family and commonly known as wood apple. In India, *A. marmelos* or bael plant is grown as a temple garden plant and the leaves are used to pray Lord Shiva; apart from India which is also found in Thailand, and various south eastern Asian countries. *A. marmelos* is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems [2]. Aegle marmelos is a medicinal plant belonging to the family. All parts of *Aegle marmelos* has many medicinal properties like astringent, aphrodisiac, demulcent, haemostatic, antidiarrheal, antidysenteric, antipyretic, antiscourbutic, and as an antidote to snake venom [3]. Various parts of the plant like the leaves and ripe fruit are used mostly in remedies for dysentery, diarrhoea, and diabetes mellitus [4].

Many of the researchers have reported the pharmacological activities of different parts of *Aegle marmelos* which includes antioxidant, free radical scavenging antibacterial, antiviral, anti-diarrheal, hepatoprotective, anti-diabetic, cardioprotective, gastroprotective, anti-ulcerative colitis and radioprotective effects. As at present scenario, only a few articles are available on the phytochemical and pharmacological values of fruit pulp of *A. marmelos*, the present review attempts to summarize the different bioactive compounds present in the fruit pulp of the plant which contributes to its medicinal properties in curing different aliments.

2. Materials and Methods:

Materials:

The Bael fruit was collected from village Simlaguri (Dotma), Kokrajhar, Assam, India. Fruits were washed and shade dried naturally for a few days and after that stored in an oven and then grind. Fully ripe bael fruits were chosen for this study because this is what usually consumed fresh and used as a main ingredient in many food products and traditionally medicines. The pulps were removed from the ripe fruits, and were analyzed for physicochemical properties. All the chemicals are purchased from Sigma Aldrich and are pure analytical grade.

Preparation of the extracts:

After collection of the fruits, the pulp part was gathered and air dried, powdered and then subjected to solvent extraction using standard protocol. Three solvents (hexane, ethyl acetate, ethanol were used. The plant material (fruit pulp powder) was put in a conical flask and the solvent hexane was added till the material submerged. Then the flasks were kept for shaking for a period of twenty four hours at room temperature in an orbital shaker. At the end of twenty four hours, the content was filtered and concentrated using rotary evaporator. The dried mass thus obtained was kept in refrigerator by covering with aluminium foil and the process was repeated for three consecutive times. Then the material which was left in filter paper was kept for extraction by adding solvent (ethyl acetate), ethanol and 50% ethanol-water using the same procedure. The extract was concentrated using rotary vacuum evaporator to get dry powder. The crude extracts thus obtained were used for further investigation of phytochemical Screening.

Qualitative phytochemical analysis:

All the plant extracts of fruit pulp of *Aegle marmelos* were tested for the presence of carbohydrates, saponins, alkaloids, flavonoids (phenolic compounds) by following the methods-

Tests for Saponins: 0.2 gm of plant extract was taken in test tubes and 5 mL of distilled water was added to each and heated to boil. Occurrence of frothing (appearance of creamy mass of small bubbles) in the test tubes indicated the presence of Saponin [5].

Test for Flavonoids: 2mL of plant extracts were taken in separate test tubes and diluted Sodium hydroxide was added followed by addition of diluted Hydrochloride. Yellow solutions were observed turning colorless which indicated the presence of flavonoids [5].

Test for Phenol: 1mL of aqueous /alcoholic solution was taken in test tubes and 3-4 drops of ferric chloride reagent was added. Violet color was obtained indicating the presence of phenols in the test samples [5].

Test for Carbohydrates: Crude extract was mixed with few drops of 1-naphtol solution in alcohol and the Concentrated H_2SO_4 was added from the side of the test tube. Violet ring formed at the junction of two liquids showed the presence of carbohydrates [6].

Test for Alkaloids: Crude extract was mixed with 2 ml of 1% HCL and heated gently. Mayer's and Wagner's reagent were added to the mixture. Appearance of cream color precipitates with Mayer's reagent and reddish brown precipitates with Wagner's reagent indicates the presence of alkaloids [6].

Test for Tannins: Crude extract was mixed with 2 ml of neutral $FeCl_3$. A dark green coloration indicated the presence of Tannins [6].

Table 1: Qualitative phytochemical analysis of different solvent extract of fruit pulp.

Sl. No.	Phytochemical compounds	Hexane	Ethyl Acetate	Ethanol	50% Ethanol-water
1	Saponins	+	+	+	+
2	Flavanoids	+	+	+	+
3	Phenols	-	-	+	+
4	Carbohydrates	+	+	+	+
5	Alkaloids	+	+	+	+
6	Tannins	-	-	+	+

+ Presence of constituents, - absence of constituents

Quantitative phytochemical analysis:

Determination of moisture content:

The moisture content was determined following AOAC method. Briefly, 5 g of fresh sample was completely dried in a hot air oven at 105 degree centigrate for 3 h, cooled in desiccator, weighted and the moisture content was calculated by the following formula [7].

Moisture (%) = Fresh weight of the sample (Fresh weight - Dry weight) × 100 %

Determination of crude protein:

Crude protein was determined by Kjeldhal method following the AOAC method. 1 g of the sample was digested with 20 mL concentrated H_2SO_4 and Kjeldhal catalyst (9 parts of K_2SO_4 and one part of $CuSO_4$) in a digestion chamber until it becomes clear. The blank test was performed without the sample. After digestion, it was distilled in Kjeldhal distillation chamber (Buchi Kjelflex K-360). The evaporated ammonia was condensed and then titrated against the known concentration (0.1 N) of HCl. The concentration of nitrogen was calculated by the following formula [7].

Nitrogen (%) = (A - B) x N of HCl x 14 Weight of the sample x 1000, where, A = Volume (mL) of (0.1 N) HCl used in sample titration, B = Volume (mL) of (0.1 N) HCl used in blank titration, 14 = Atomic weight of Nitrogen.

The nitrogen content thus determined was multiplied by a protein conversion factor of 6.25 to get the amount of crude protein.

Determination of DPPH radical scavenging assay:

Radical scavenging activity of plant extracts against stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm wavelength. Radical scavenging activity of extracts was measured by a standard method. Two microliters of each sample, prepared at various concentrations (10, 20, 50, 100, 250 mg/mL), were added to 2 mL of 0. 2M DPPH solution. The mixture was shaken and allowed to stand for 30 min at 20°C, and then the absorbance was measured at 517 nm with UV-VIS spectrophotometer. The percentage of inhibition activity was calculated by the following equation: DPPH scavenging effect(%) = [($A_{control}$ - A_{sample}) / $A_{control}$ x 100], where $A_{control}$ is the initial concentration of the stable DPPH radical without the test compound and A_{sample} is the absorbance of the remaining concentration of DPPH in the presence of methanol. IC_{50} values (mg/mL) were determined from a plotted graph of scavenging activity against the concentrations of the *Aegle marmelos* fruit extracts, where IC_{50} is defined as the total amount of antioxidant necessary to decrease the initial DPPH radical concentration by 0% [8].

Table2: In vitro radical scavenging effect of Aegle marmelos fruit pulp by DPPH method.

Concentration	10 micro gm/L	20 micro gm/L	40 micro gm/L	80 micro gm/L	100 micro gm/L	200 micro gm/L	IC_{50} micro gm/L
Ethyl acetate extract	57	114	228	456	912	1140	317.4074
Ethanol extract	62	124	248	496	992	1240	53.5684
Ascorbic acid	50	100	200	400	500		53.5684

Standard graph for DPPH radical scavenging method:

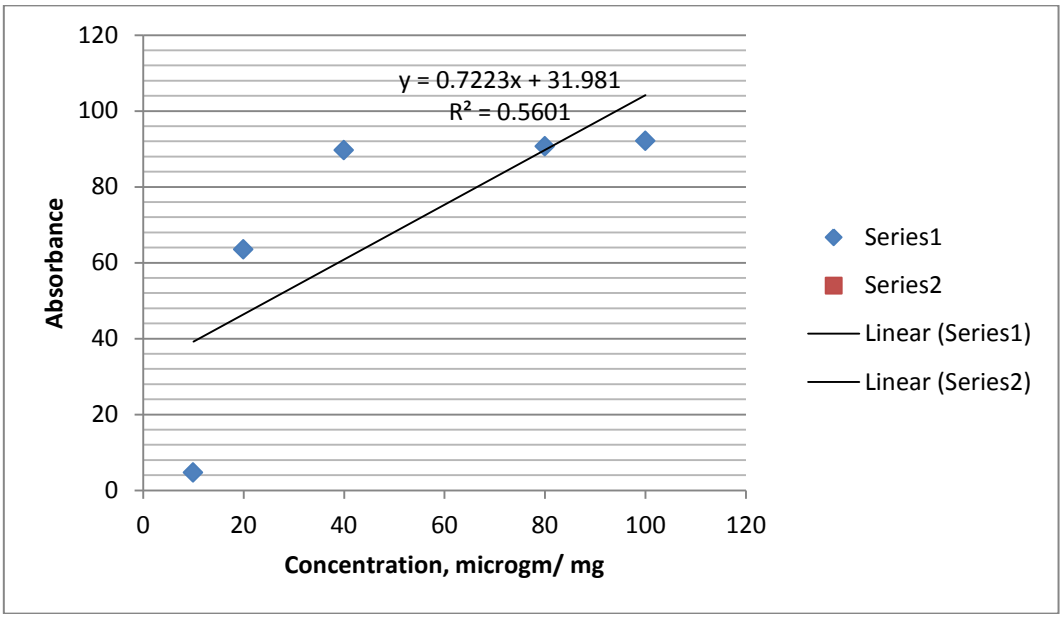

Figure 1: Radical scavenging activity of ascorbic acid.

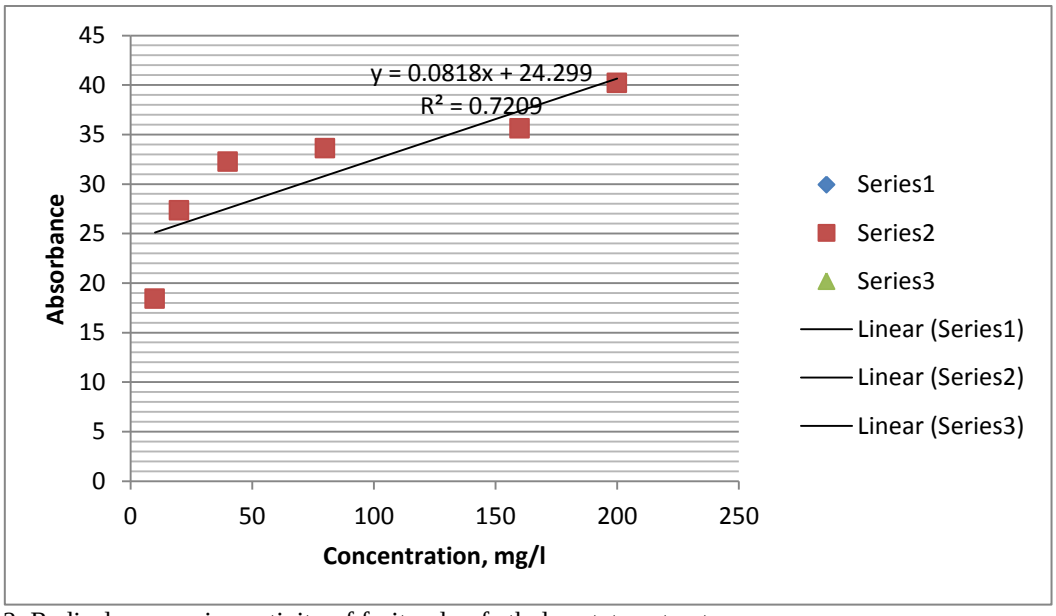

Figure 2: Radical scavenging activity of fruit pulp of ethyl acetate extract.

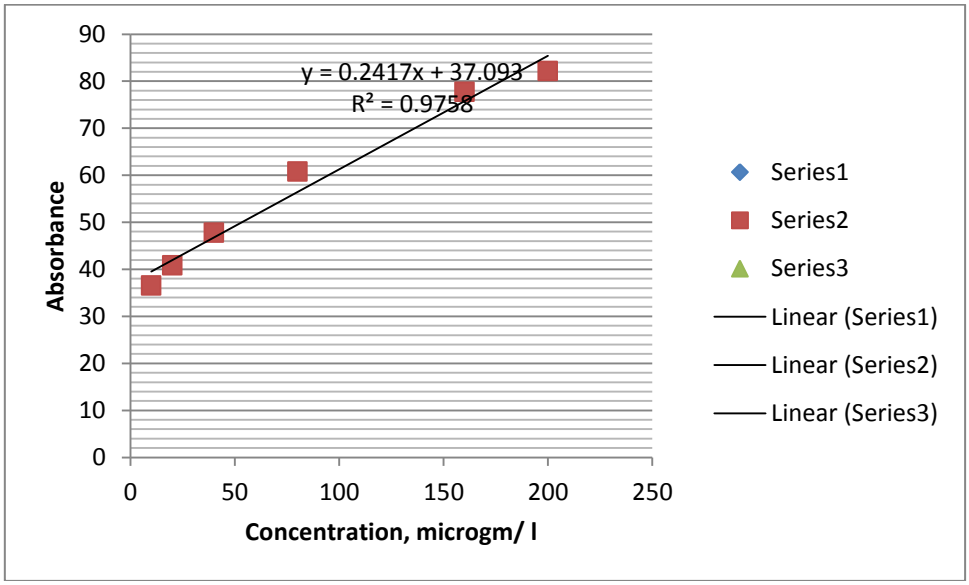

Figure 3: Radical scavenging activity of fruit pulp of ethanol extract.

The Total Phenolic content:

Total phenolic compounds of fruit extracts were determined by Folin-Ciocalteu Method. For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2, and 0.3 mg/mL methanolic gallic acid solution was mixed with 5 ml of Folin-Ciocalteu reagent (10 times diluted) and 4 ml of sodium carbonate (75 g/L). The absorbance at 765 nm was measured after 1 h at 20◦C and the calibration curve was drawn. Then 1 mL of methanolic fruit extracts (50 mg/ml fresh weight tissue) was mixed to the same reagent and the mixture was incubated for 1 h in room temperature. After 1 h, the absorbance was measured at 765 nm [9].

Table 3: Total phenolic contents in the absolute alcohol and ethyl acetate of fruit pulp.

Fruit pulp extracts (samples)	Concentration of Phenolic (microgm GAE/mg)
Absolute alcohol extract	8.428
Ethyl acetate extract	10.285

Standard calibration curve for phenolic content of ethyl acetate and ethanol extract:

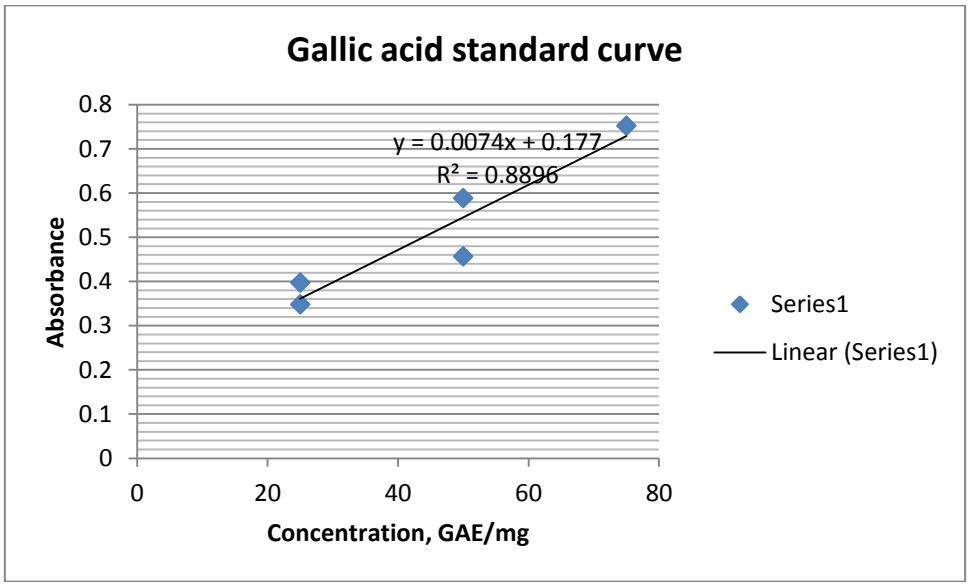

Figure 4: standard calibration curve of gallic acid of ethyl acetate extract.

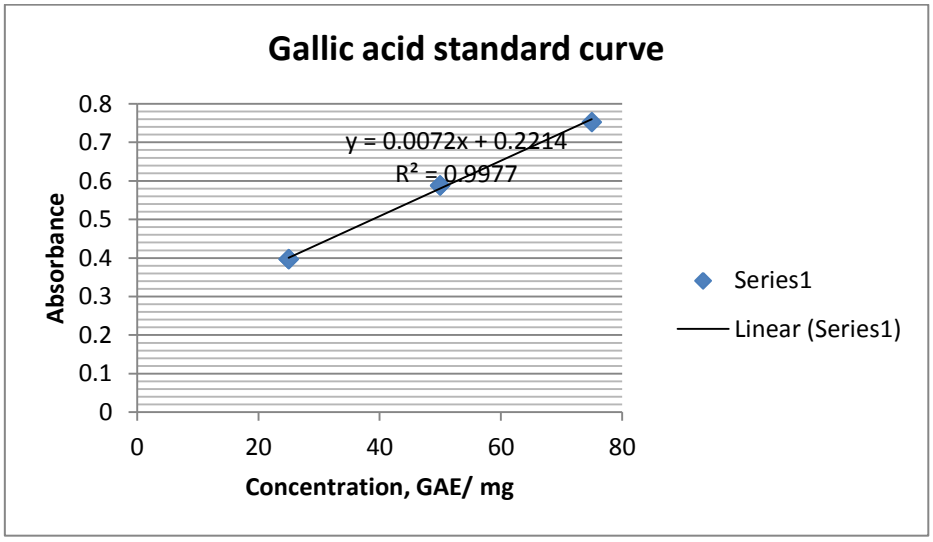

Figure 5: Standard calibration curve of gallic acid of ethanol extract.

The amount of phenolic content in alcohol extract was found to be 8.425 micro gm GAE per mg of extract. Similarly, the total amount of phenolic content in the ethyl acetate extract was found to be 10.285 micro gm GAE/mg. From study we have seen that amount of phenolic content is more than in other extracts.

The total Flavanoid content:

A spectrophotometric aluminum chloride method was used for flavonoid determination. Each of the fruit methanol extracts (0.5 mL of 100 mg/mL FW) were separately diluted with 4 mL of double-distilled water. Then the diluted fruit extracts were mixed with 5% (0.3 mL) $NaNO_2$ and 10% aluminum chloride was then added with the reaction mixture. After 6 min, 2 mL (1.0 M) NaOH and 2.4 mL double distilled water were added and mixed well. Thereafter, absorbance was measured at 510 nm by a spectrophotometer. Standard solution of quercetine (0–500 mg L^{-1}) was used as the calibration curve [10].

Table 4: Total Flavanoid contents in the absolute alcohol and ethyl acetate of fruit pulp.

Fruit pulp extracts (sample)	Amount of flavanoid (microgm QE/ mg)
Absolute alcohol extract	5.5
Ethyl acetate extract	4

Standard calibration curve for flavanoids of ethyl acetate and ethanol extract:

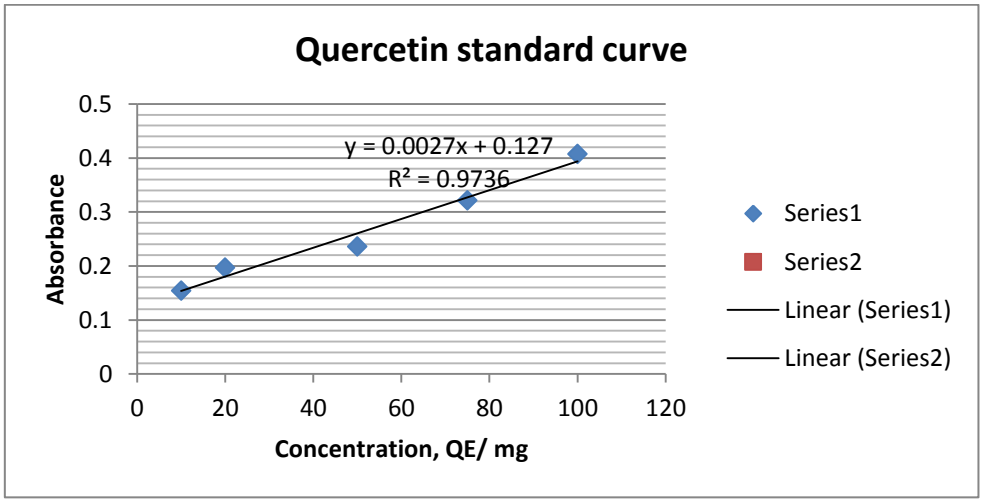

Figure 6: Standard calibration curve of quercetin for ethyl acetate extract.

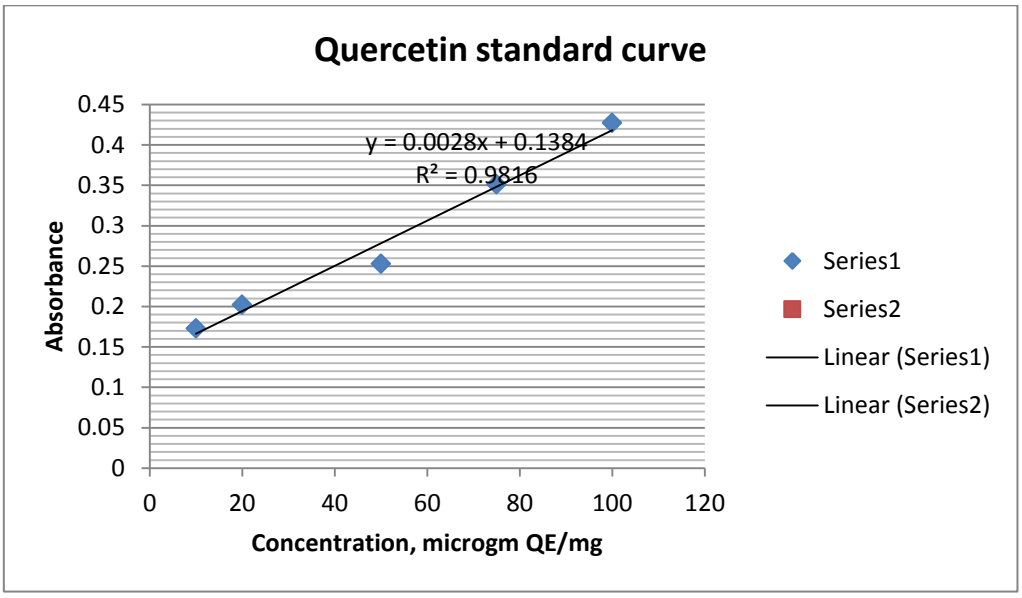

Figure 7: Standard calibration curve of quercetin for ethanol extract.

We have found, the amount of flavanoids in absolute alcohol extract is 5.5 microgm QE/ mg and with Ethyl acetate extract is found to be 4 microgm QE/ mg. Hence, the amount of flavanoids in alcoholic extract is more than that of ethyl acetate extract.

3. Conclusion:

Based on the result in the study, it was concluded that extracts of *Aegle marmelos* fruit pulp available in kokrajhar area were found to be a good natural antioxidant. From the study we can say that, the amount of phenolic content is more in ethyl acetate content than that of other extracts. Also amount of phenolic content is more in both alcohol and ethyl acetate extract compared to that of the amount of flavanoids content. Further scope of studies has become a challenge to identify specific active components of this plant for the significant antioxidant effect as well as other medicinal values.

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