International Journal of Aquatic Science
ISSN: 2008-8019
Vol 12, Issue 02, 2021

Determination of In-Vitro Antioxident And In-Vitro Antidiabetic Activity of Active Fraction From Aegle Marmelos Leaves Extract

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Abstract: Aegle marmelos leaves has been used as a remedy for gastrointestinal infections, antidiarrheal, anti-inflammatory and antioxidant activity of human beings. Extraction was obtained using methanol and fractionation in column chromatography with different solvent followed with: beginning with chloroform, ethyl acetate, methanol and water. The invitro antioxidant activity, total flavonoid content and Total polyphenol content seven fraction of Aegle Marmelos leaf extract was determine by using spectrophotometric method. The all fraction obtained from extract were based on solvent concentration for seven fractions. The total antioxidant capacity of the fractions was measured in mg/ml of ascorbic acid, with a range of 35 to 132 mg/ml, total flavonoid content of the fractions was expressed of quercetin equivalent (QE) ranging between 47.5 – 83.5 mg/ml and total phenolic content of the fractions were expressed of Gallic acid equivalent (GAE) ranging between 96.3 – 181.5. All the fractions, it was observed that water fraction having better antioxidant property, so the it was preferred for invitro diabetic activity. The Percentage inhibition of Acarbose was found to be 33.23 µg/ml and fraction of ethanolic extract of Aegle marmelos percentage inhibition was to be 156.84 µg/ml.

Keywords: Active fraction, Aegle marmelos, in-vitro antioxidant activity and in-vitro antidiabetic activity.

1. INTRODUCTION:

Diabetes mellitus is a serious complex multifactorial disorder characterized by hyperglycemia (very high blood glucose level) and glucose intolerance, either because of a relative lack of insulin production or because insulin's ability to promote glucose absorption is diminished. If left untreated, it might lead to major problems. These complications include hyperlipidemia (abnormal high level of lipid in the blood), oxidative stress, and enzymatic glycation of protein. [1] Antioxidative systems in human bodies, both enzymatic and non-enzymatic, help to reduce the production of reactive oxygen species, which are linked to a variety of degenerative disorders, including diabetes. [2] People with diabetes have elevated blood glucose levels as a result of insulin insufficiency.[3] However, many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects. [4] These constraints have encouraged researchers to investigate treatment techniques incorporating the use of medicinal plants that have been shown to be cost-effective antidiabetic medicines with fewer adverse effects. [5] In countries like India, Due to the relatively expensive cost of allopathic remedies,

it is advantageous to use a variety of indigenous plant remedies. [6] Herbal medicines are gaining popularity due to their efficacy, low cost, and clinical expertise with minimal adverse effects. Even when the biological active ingredients of herbal medications or extracts are unknown, they are commonly recommended. Even the World Health Organization approves the use of plant drugs for different diseases including diabetes mellitus. [7] Plants are a decent drug source and majority of drugs are derived from them, either directly or indirectly. The ethnobotanical information reported about 800 plants may possess antidiabetic potentials. [8]
It is indigenous to India and is used in folk medicines.[9] Ayurvedic practitioners employ practically all of the plant's components, but the fruits have the highest therapeutic potential. Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental and chemical factors which cause generation of various reactive free radicals and subsequent damage to macromolecules like DNA, Proteins and Lipids. No specific scientific evaluation of antioxidant activity of *A. marmelos* fruit pulp has been reported so far.[10-12]

2. MATERIAL AND METHODS:

2.1. Collection of plants leaves:

The leaves of *Aegle Marmelos* was collected from a thick forest in western District in Eastern Odisha, India. The leaves were washed with fresh water and dried in shade away from the sunlight for few days. The dried leaves were grinned in mechanical grinder.

2.2. Preparation of herbal extract:

Preparation of plant extract of *Aegle Marmelos* was carries out by the maceration method. The grinned leaves were filled in a glass jar with air tight cap. The sufficient amount of methanol was poured into the glass jar as a solvent. This glass jar was kept aside from sunlight for various days. After 7 days the whole solvent was filtered by a muslin cloth or filter paper and allow to dried to collect the semisolid dried extract of plant leaves. [13]

Fractionation of ethanolic extract of *Aegle Marmelos and Pedalium murex*

Ethanolic extract was subjected to column chromatography to separate it component fraction into the extract. Silica gel are used in the packing the column while different solvent combination based on polarity incising were used the mobile phase as described as Yakubu et al., [14]

Packing of column:

In the packing of the column, With the help of glass rod, the lowest half of the glass column was loaded with glass wool. To create the slurry, 75g of silica gel (G60-200 mesh size) was dissolved in 180 ml of pure chloroform. The silica gel-packed chromatographic column (30mm diameter by 40 cm height) was permitted free flow of the solvent into a conical flask below. When the solvent drained easily without bringing the silica gel or glass wool into the tap, the setup appeared to be in order. The tap was locked at the end of the packing operation, and the column was given 24 hours to settle before the clear solvent on top of the silica gel was allowed to drain through the silica gel meniscus.

Elution:

The ethanol extract (5g) was dissolved in 5 ml absolute ethanol and the solution was applied unto a chromatographic column. The extract was eluted with a polarity-gradient solvent system that included chloroform, ethyl acetate, methanol, and distilled water. The following ratio of solvent combination was consecutively used in the elution protocol:

i) Chloroform: ethyl acetate 100:0, 50:50, 0:100.
ii) Ethyl acetate: methanol 50:50, 0:100.

iii) Methanol: water 50:50 and 0:100.

Using a separator funnel, a measured volume (500 ml) of each solvent combination was put onto the column each time. The eluted fractions were composed in aliquots of 500 ml in fraction collection tubes.

Determination of Total Flavonoid Content (TFC):

The total flavonoids content was determined using the aluminium chloride technique [15,16]. In 10 ml methanol, 10 mg quercetin was dissolved, and different aliquots of 5- 25g/ml were made in methanol. Total flavonoids content was expressed as mg/ml quercetin equivalent (QE). The concentration of flavonoids in the sample was estimated using the calibration curve.

Determination of Total Phenol Content (TPC):

The modified folin-ciocalteu technique was used to determine the total phenol concentration of the extract [17]. The total polyphenol component was measured colorimetrically at 765 nm using the Follin-Ciocalteu reagent and expressed as gallic acid equivalent, as reported by Lachman et al (GAE). The reactions were carried out in triplicate, and the absorbance of the sample was measured at 765nm against the reagent blank.

Determination of Total Antioxidant Capacity (TAC)

The scavenging action of the plant extracts and the resulting fractions from ethanol extract on 1,1-diphenyl-2- picrylhydrazyl (DPPH) was determined calorimetrically at 517 nm using Ascorbic acid as standard according to the means represented by Singleton et al.,[18]. Each fraction's absorbance was measured in triplicate at 517nm. Total antioxidant capacity (TAC) was calculated as mg/ml of ascorbic acid equivalent using the regression equation from calibration curve.

2.3. Inhibition of α-amylase Enzyme [19]

α-amylase (0.5 mg/ml) was mixed with the sample at various concentrations (100-500 µg/ml) to which 1% of starch solution and 100 µl of 0.2 mm of phosphate buffer (pH -6.9) were added. The reaction was allowed to run for 5 minutes at 37°C before being stopped with the addition of 2 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 minutes at 100°C before being diluted in an ice bath with 10 ml of distilled water. α-amylase activity was determined by measuring color intensity at 540 nm in spectrophotometer.

2.4. Inhibition of α-glucosidases Enzyme [20]

In order to test the inhibitory action, 1 ml of starch solution (2 percent w/v maltose) was incubated with 0.2 M tris buffer (pH 8) and different concentrations of sample (100-500 mg/ml). For 10 minutes, the reaction mixture was incubated at 37°C. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. The reaction was then stopped by adding 2 ml of 6 N HCl. The colour intensity was measured using a spectrophotometer at 540 nm.

The results were expressed as % inhibition using the formula:

$$\% \text{ Inhibitory activity} = \frac{(Ac - As)}{Ac} \times 100$$

Where, Ac is the absorbance of the control and

As is the absorbance of the sample.

The inhibitory concentration (IC50) value is defined as the concentration of inhibitor required to inhibit 50% of its activity under the test circumstances. The IC50 values were computed using logarithmic regression analysis from the mean inhibitory values based on plots of inhibition zone vs log inhibition efficiency.

Statistical Analysis
All determinations were done in triplicate and values are expressed as the mean ± standard error of the mean. The result is also expressed as IC50 value. IC50 value was calculated using regression analysis.

3. RESULTS AND DISCUSSION:

Results:
The Total Flavonoid Content (TFC), Total Polyphenols Content (TPC) and Total Antioxidant Capacity (TAC) revealed that the methanol: water fraction has the highest total antioxidant activity and then continues as shown in the table 1.

Table 1: Total flavonoid content, Total polyphenol content and total antioxidant capacity of Aegle Marmelos

Fractions	Solvent System	TFC (mg/ml)	TPC (mg/ml)	TAC (mg/ml)
1	Chloro.: Eth. Acetate (100:0)	47.5	108.2	48
2	Chloro.: Eth. Acetate (50:50)	58.3	96.3	35
3	Eth. Accet. :Etha. (100:00)	76.5	116.5	47
3	Eth. Accet. :Etha. (50:50)	78.2	128.3	53
5	Etha. : Meth (100:0)	62.4	119.5	58
6	Etha. : Meth. (50:50)	71.4	123.2	48
7	Meth: water (100:00)	78.2	133.4	42
8	Meth.: water (50:50)	83.5	181.5	132

The correleation between TFC vs TPC, TPC vs TAC, TAC vs TFC for Aegle marmelos methanolic leaf extract.
From the results of above data that all the all the correlations were positive correlation but flavonoid content is the better (Figure 3) correlation with Total antioxidant capacity (TAC). And also positive correlation was observed in other parameter show in figure 1 and figure 2.

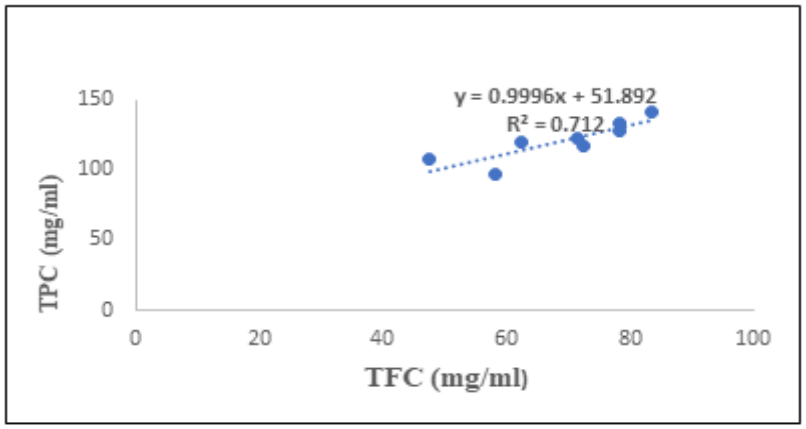

Figure 1: Correlation between total flavonoid content and total polyphenol content of fraction obtain from methanolic extract of *Aegle marmelos* leaf extract.

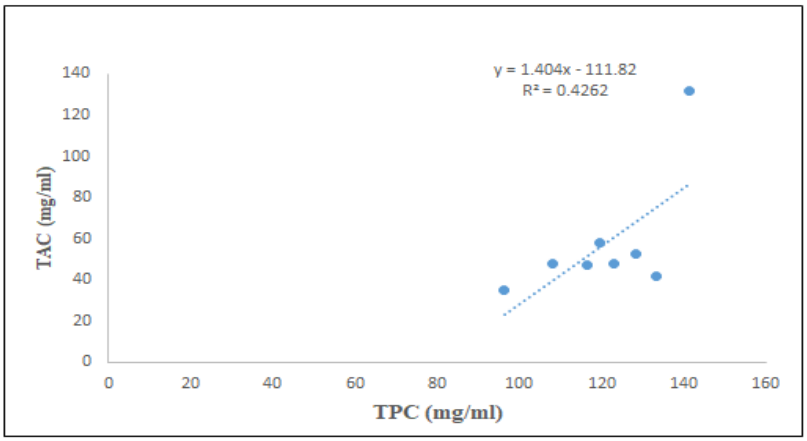

Figure 2: Correlation between total polyphenol content and total antioxidant capacity of fraction obtain from methanolic extract of *Aegle marmelos* leaf extract.

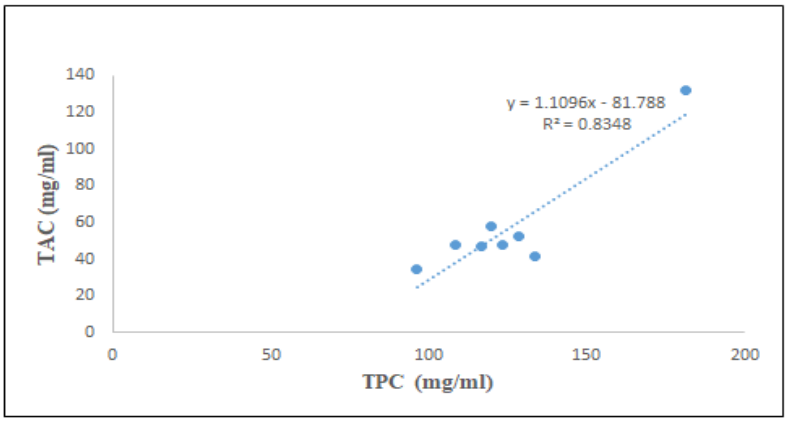

Figure 3: Correlation between total antioxidant capacity and total flavonoid content of fraction obtain from methanolic extract of *Aegle marmelos* leaf extract.

Table 4: *In vitro* antidiabetic studies of fraction obtain from methanolic extract of *Aegle marmelos* leaf extract

S. No.	**Concentration (µg/ml)**	**% Inhibition**	
		Acarbose	**fraction *of* *Aegle marmelos***
1.	10	33.86	11.58
2.	20	42.36	16.48
3.	40	52.73	19.73
4.	60	68.73	24.38
5.	80	83.45	29.29
6.	100	91.13	36.62
IC_{50}		**33.23**	**156.84**

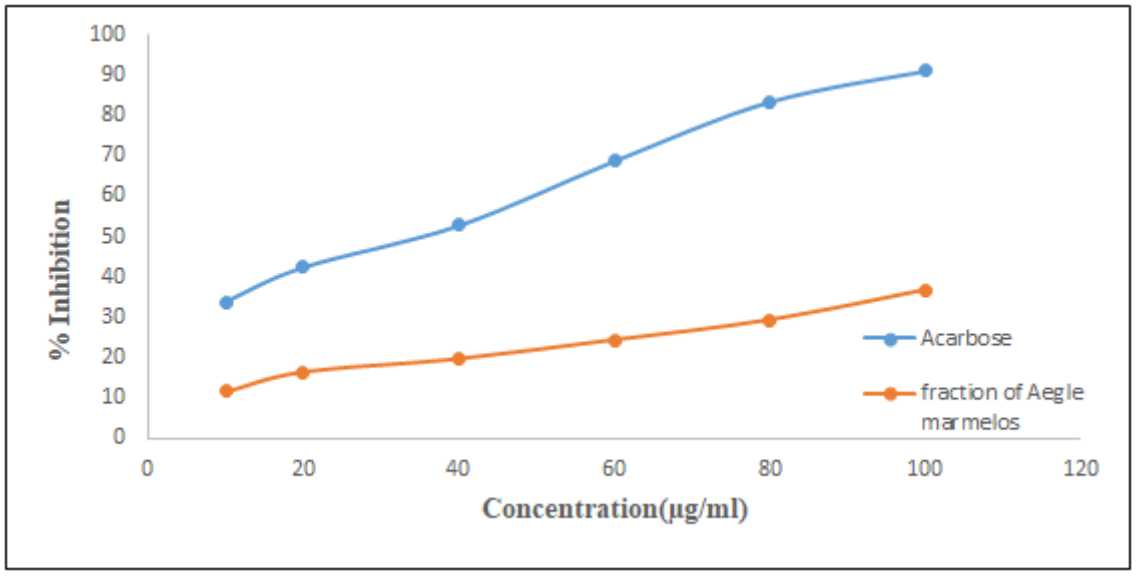

Figure 1: *In vitro* antidiabetic studies of fraction obtain from methanolic extract of *Aegle marmelos* leaf extract

4. DISCUSSION:

As per the above result it was shown that there is strong relationship between total phenolic content and antioxidant capacity on the plants that is observed in *Aegle marmelos* leaf extract (R^2 = 8.834) which could be based on total antioxidant capacity. And it was observed also a strong positive correlation (R^2 =0.426) which is based on the total phenolic content present in total antioxidant when correlated. Although it is consistent, the total antioxidant capacity of ethanol extract of *Aegle marmelos* leaves depend on the polarity of the eluting solvent.

The total antioxidant of *Aegle marmelos* leaves ranged from 47.5-83.5 mg/ml Quercetin equivalent and total phenol capacity ranged from 96.3-181.5 mg/ml GAE (table 1) from this report the total flavonoid capacity of Ethanol extract of *Aegle marmelos* may be responsible for Antioxidant activity since a strong positive correlation (R^2= 0.8348) is observed with the total antioxidant capacity as shown in figure 3.

In the current study there was a strong relationship between Total antioxidant and total flavonoid content of *Aegle marmelos* leaves. As a result, the overall antioxidant capability of a fraction is heavily influenced by its flavonoinds concentration.

α-amylase inhibitory activity was used to test the anti-diabetic effect of *Aegle marmelos* leaves in vitro. The percentage inhibition of acarbose was determined to be 33.23 g/ml, and the percentage inhibition of ethanolic extract of *Aegle marmelos* was noted in table 4 and figure 1.

5. CONCLUSION:

According to the findings of this investigation, the water fraction demonstrated the maximum antioxidant activity, total phenol capacity, and total flavonoids concentration of Aegle marmelos leaves ethanol extract. Hence, there was strong positive correlation between the TAC and the TPC, indicating that the flavonoids content of the extract are to a larger extent responsible for the elicited antioxidant effects of the extract. Aegle marmelos leaves were tested for anti-diabetic effectiveness in vitro employing α-amylase inhibitory activity in comparison to conventional Acerbose.

6. REFERENCE:

[1] Al-Hourani HM, Atoum MF. Body composition, nutrient intake and physical activity patterns in young women during Ramadan. Singapore Medical Journal. 2007; 48(10): 906–910.
[2] Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012;2(4):320-330. doi:10.1016/S2221-1691(12)60032.
[3] Gupta P, Bala M, Gupta S et al., Efficacy and risk profile of anti-diabetic therapies: Conventional vs traditional drugs—A mechanistic revisit to understand their mode of action. Pharmacological Research. 2016; 113: 636–674.
[4] Atanasov AG, Waltenberger B, Pferschy-Wenzig EM. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnology Advances. 2015; 33 (8): 1582–1614.
[5] Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of Type 2 diabetes: Indian scenario. Indian J Med Res. 2007;125(3):217-30.
[6] Ghazanfar H, Rizvi SW, Khurram A, Orooj F, Qaiser I. Impact of insulin pump on quality of life of diabetic patients. Indian J Endocrinol Metab. 2016;20(4):506-11.
[7] Rastogi A, Shankar S, Mahalingam G. Phytochemical screening, anti-oxidant activity and in vitro anti-diabetic activity of aqueous, methanolic, ethanolic and chloroformic extracts of hygrophilaauriculata. Int J Pharm Pharm Sci. 2014;6(5):557-60.
[8] Murty R, Kadali SLDV, Das MC, Vijayaraghavan R, Shanmukha I. In vitro evaluation of antidiabetic activity of aqueous and ethanolic leaves extracts of Chloroxylon swietenia. National Journal of Physiology. Pharmacy and Pharmacology. 2017:7:5.
[9] Nagar S, Kumar M, Kumatkar RB, Sharma JR, Sing S. Evaluation of bael (*Aegle marmelos* Corr.) germplasms for seed and qualitative characters under semi-arid conditions of Haryana. International Journal of Pure & Applied Bioscience. 2017; 5: 436–442.
[10] Roy SK, Sing RN. Bael fruit (*Aegle marmelos*): a potential fruit for processing. Economic Botany. 1979; 33(2):203–212.
[11] Singhal VK, Salwan A, Kumar P, J Kaur. Phenology, pollination and breeding system of *Aegle marmelos* (Linn.) correa (Rutaceae) from India. New Forest. 2011; 42(1): 85–100.
[12] Mukherjee PK. Quality control of herbal drugs: an approach to evaluation of botanicals. New Delhi: Business Horizons Pharmaceutical Publishers; 2002.
[13] Jayaprakasha GK, Jaganmohan RL, Sakariah KK. Antioxidant activities of flavidin in different *in vitro* model systems. Bioorg Med Chem. 2004;12(19):5141–5146.
[14] Yakubu OE, Nwodo OFC, Joshua PE, Ugwu MN, Odu AD and Okwo F. Fractionation and determination of total antioxidant capacity, total phenolic and total flavonoids contents of aqueous, ethanol and n-hexane extracts of Vitex doniana leaves. Afr. J. Biotechnol. 2014; 13 (5): 693-698.
[15] Parkhe G and Bharti. Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of Vitex trifolia Linn, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):705-707
[16] Chang CC, Yang MH, Wen HM, Chm JC. Estimation of total flavonoids in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10: 178-182.

[17] Parkhe G and Bharti D. In Vitro Antioxidant Activity, Total Phenolic and Flavonoid Contents of Hydroalcoholic Extract of Leaves of Lagerstroemia ParvifloraRoxb. Journal of Drug Delivery and Therapeutics. 2019; 9: 708-711.
[18] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenol and other oxidative substrates and antioxidants by means of Folin-ciocalteu reagent. Methods Enzymol. 2002; 299: 152-178.
[19] Pant G, Sai K, Babasaheb S, Reddy R, Sibi G. In vitro α-amylase and α-glucosidase inhibitor activity of abutilon indicum leaves. Asian J Pharm Clin Res. 2013;6(5):22-4. 20.
[20] Vennila V, Pavithra V. In-vitro alpha amylase and alpha glucosidase inhibitory activity of various solvent extracts of Hybanthus enneaspermus Linn. World J Pharm Pharm Sci. 2015;4(4):1425-37.