

FORMULATION AND ASSESSMENT OF HERBAL FORMULATION CONTAINING EXTRACTS OF AEGLE MARMELLOS FOR ANTIOXIDANT ACTIVITY

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Abstract:

The present study investigates the antioxidant potential of a herbal formulation incorporating the extract of *Aegle marmelos*, a medicinal plant traditionally renowned for its therapeutic properties. The formulation was developed using optimized extraction techniques to ensure maximum retention of bioactive compounds. The antioxidant activity of the *Aegle marmelos* extract and the formulated product was evaluated through various *in vitro* assays, including DPPH radical scavenging. Methanol was used as the solvent in the soxhlation process to extract compounds from *Aegle Marmelos*, the extract was assessed for antioxidant activity. After the acute oral toxicity research was finished, the *in vivo* pharmacological activity was assessed for its ability to reduce oxidant levels. The extract was compressed into tablets, and the tablets quality was assessed. The isolated compounds from the extract of *Aegle Marmelos* showed antioxidant effect but the potency as less as compared to the standard ascorbic acid 5 mg/kg. The quality of tablets compressed containing the extract found to show promising results.

Keywords: *Aegle Marmelos*, Soxhlation, antioxidant activity, DPPH

Introduction

Now days, natural products are an integral part of human health care system because, there is accepted fear of toxicity and side effects of modern drugs. There is also a realization that natural drugs are safer and allopathic drugs are often ineffective. In the last few decades there has been an exponential growth in the field of herbal medicine.(1) It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. The Indian contribution in international herbal market has emphasized on novel research for capturing as well as to remain in the market, phytochemical standardization of herbal drugs and highly processed materials in herbal formulation.(2) The importance and challenges of conducting clinical research in herbal drugs, simple bioassays for biological standardization, pharmacological & toxicological evaluation, toxic herbal drugs in use and various animal models for toxicity & safety evaluation were dealt in detail by various experts in the field. (3)

Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates, is implicated in the pathogenesis of numerous chronic diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders.(4) Antioxidants, which can neutralize ROS and mitigate oxidative damage, are thus of significant interest in the prevention and management of these

conditions. While synthetic antioxidants are available, their potential side effects and toxicity have spurred a growing interest in natural alternatives, particularly those derived from medicinal plants.(5)

Aegle marmelos (L.) Correa, commonly known as bael, is a tree native to the Indian subcontinent and Southeast Asia. It has been traditionally used in Ayurveda and other systems of traditional medicine for its diverse therapeutic properties, including antidiabetic, anti-inflammatory, and hepatoprotective effects. Recent scientific investigations have highlighted its rich phytochemical profile, which includes flavonoids, tannins, phenolic acids, and alkaloids, compounds known for their potent antioxidant activities.(6,7)

Despite the promising antioxidant potential of *Aegle marmelos*, there is a paucity of research focusing on its application in formulated herbal products designed to harness and enhance these properties.(8,9) This study aims to bridge this gap by formulating a herbal product containing *Aegle marmelos* extract and evaluating its antioxidant efficacy through various in vitro assays. By doing so, the research seeks to establish a scientific basis for the use of *Aegle marmelos* in antioxidant therapy and to explore its potential as a natural alternative to synthetic antioxidants.(10,11)

2. Materials and Methods:

2.1 Collection and Authentication of Plant

The whole plant or their parts were collected and authenticated by an authority in plant taxonomy. The identified and authenticated species were collected in sufficient quantity, dried and powdered for further studies.(12,13)

2.2 Drying and Size Reduction of Plant Material

The *Aegle Marmelos* was pulverized to moderately coarse powder. The coarse powder of rhizomes was passed through sieve No. 16 to maintain uniformity and stored in cool and dry place for further study.(14,15)

2.3 Preparation of *Aegle Marmelos* Extract

a) Extraction of *Aegle Marmelos*

Soxhlation method was used for extraction of *Aegle Marmelos* using methanol as the solvent for extraction, 250 ml of solvent was placed in thimble with crushed leaves and inserted in soxhlet extractor. The side arm was covered with glass wool. The process should run for a total of 16 hours. Extract was dried on Rota evaporator then the extract was collected.(16,17)

2.4 Fractionation of methanolic extract by silica gel column:

Methanolic extracts were fractionated with column chromatography using chloroform-methanol eluents in a gradient way, ranging from (80:20), (50:50) and (25:75) to categorize the compounds contained in the methanol extracts based on their polarity. 9 different fraction were extracted with

varying range of solvent used in extraction. Looking at the amount of fraction, F1, F3, F5, F7 and F8 was selected. The quantity of F2, F4 and F9 was very less amount of fraction, that's why it was removed. The isolated compounds obtained after fractionation are mentioned in table 2.1.(18,19)



Figure 2.1: Soxhlet extraction of *Aegle Marmelos*

Table 2.1: Isolation of various extract of *Aegle Marmelos* Fraction

Fraction	Solvent system	Amount (gm)	% of fraction
F-1	Chloro.: Eth. Acetate (80:20)	1.10	13.3
F-2	Chloro.: Eth. Acetate (50:50)	0.28	5.3
F-3	Chloro.: Eth. Acetate (25:75)	0.29	4.1
F-4	Eth. Accet. :Meth. (80:20)	0.95	11.1
F-5	Eth. Accet. :Meth. (50:50)	0.41	5.3
F-6	Eth. Accet : Meth (25:75)	0.30	5.1
F-7	Meth. : water. (80:20)	1.95	22.9
F-8	Meth: water (50:50)	1.45	18.3
F-9	Meth.: water (25:75)	0.18	3.1

2.5 DPPH radical scavenging assay

This spectroscopic assay uses the stable radical DPPH as a reagent. The hydrogen atom or electron denoting abilities of the compounds and some untained compounds can be measured from the bleaching of the purple-coloured methanol solution of 2-2-diphenyl-1-picrylhydrazyl (DPPH).

(20,21)DPPH scavenging activity was measured by the spectrophotometer. Prepared the different concentration of standard solutions of ascorbic acid (10- 100 µg/ml). then prepared plant extract solution in ethanol. The prepared 0.3 mM solution of DPPH in 100% ethanol. Add 3ml of the sample extract in to 1 ml of DPPH solution. The same reaction mixture without the extract sample but with equivalent amount of standard phosphate buffer should serve as control. Shake the mixture and allow to stand at room temperature for 30 min. Measure the absorbance of reaction mixtures at 517 nm.(22)

$$\% \text{ Scavenging activity} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

2.6 Reducing Power ability:

Various concentration between 10- 100 µg/ml of samples in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 1036 x g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared FeCl₃ solution (0.5 ml, 1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.(23)

2.7 Preparation of polyherbal Tablet of Methanolic fraction

Six formulations namely formulation I, formulation II, formulation III, formulation IV, Formulation V and formulation VI with varying concentrations of the active fractions were developed. According to the formulation, required quantity of each isolated fractions with potential Antidiabetic activity and other ingredients were weighed, ground separately. Then the ingredients were screened through sieve number 80. All the ingredients except talc and magnesium stearate were mixed together and milled in a mortar pestle. The milled mixture was passed through sieve number 80. Then acacia gum solution, was slowly added to the milled mixture. This powder mass was screened through sieve number 18 to obtain granules. The granules were dried at 35°C in vacuum dryer. The dried granules were passed through sieve no. 18 in order to remove bigger granules and stored in desiccators.(24) The formulation details are mentioned in table no 6.5. Power blends according to each formulation, were compressed to 500 mg tablet by using hand rotating single punch tablet presses with appropriate compression pressure.(25,26) The granules were mixed with talc which acts as lubricant, and magnesium stearate which acts as glidant, before punching. The die cavity was adjusted for required weight and the Preformulation studies for various parameters were conducted before compression of the powder blend to tablets.(27)

Table 2.2: Formulation details of polyherbal tablet”

Ingredients	Amount (mg) for one tablet					
	F1	F2	F3	F4	F5	F6
<i>Aegle Marmelos F7</i>	10	12	15	18	20	25
Starch	20	20	20	20	20	20
Magnesium Stearate	10	10	10	10	10	10
Talc	5	5	5	5	5	5
Acacia gum	5	5	5	5	5	5
Lactose	440	431	430	424	420	410

3.Evaluation of Tablets:

3.1 Diameter and Thickness:

The diameter and thickness of the tablet were measured by using a Verniercalliper. It is expressed in mm. Six tablets were selected at random from each batch, and the mean; standard deviation values were calculated. The thickness of the tablet is important in producing tablet identical in appearance. Thickness can vary with no change in weight because of the difference in density of the powders. (28)

3.2 Hardness Test:

Hardness or tablet crushing strength (the force required to break a tablet in a diametric compression) was measured using a Monsanto tester. Six tablets from each batch wereselected and evaluated, and the average value with standard deviation was recorded. The mean \pm standard deviation values of hardness were calculated.(29)

3.3 Friability Test:

Friability test is performed to evaluate the ability of the tablets to withstand abrasion in packing, handling, and transporting. The Friability of tablets was determined using a friabilator (Roche friabilator). Ten pre-weighedtablets were placed in the friabilator, operated for 4minutes at 25 rpm. After 100 revolutions, the tablets were taken out, dedusted, and reweighed. The percentage friability of tablets was measured as per the following formula.(30,31)

$$\% \text{ Friability} = (\text{Initial weight} - \text{final weight}) / (\text{Initial weight}) \times 100$$

3.4 Weight Variation Test:

Uniformity of weight test as described in the Indian Pharmacopeia was followed; a small variation in the weight of the individual tablet is liable to occur. Therefore, a little variation is allowed in the weight of tablets by the pharmacopeia. The following percentage deviation in weight variation is allowed. To study weight variation, 20 tablets of each batch were weighed using an analytical

electronic balance, and the mean weight was calculated. Not more than 2 tablets should deviate from the average weight of the tablets.(32)

3.5 Disintegration test:

The disintegration time of tablets was determined using the digital microprocessor based disintegration test apparatus (basket rack assembly, Lab India). One tablet was introduced into each tube and added a disc. The assembly was suspended in a 1000 mL beaker filled in with water. The volume of water was such that the wires mesh at its highest point (at least 25 mm) below the surface of the water, and at its lower point (at least 25 mm) above the bottom of the beaker. The apparatus was operated and maintained at $37\pm 2^{\circ}\text{C}$. The time requires to all tablets to disintegrate and pass through wire mesh was noted.(33,34)

4. Result and discussion

4.1 Antioxidant Activity:

4.1.1 DPPH radial scavenging assay

The antioxidant potential of the selected plant extracts suchas *Aegle marmelos* was investigated in comparison with the known antioxidant ascorbic acid (AA) following in vitro studies.(35, 36, 37)

Table No. 4.1Antioxidant activity in extract of *Aegle marmelos*(DPPH radial scavenging assay)

S. No.	Concentration ($\mu\text{g/ml}$)	% Inhibition	
		Ascorbic acid	<i>Aegle marmelos</i>
1	10	42.53 \pm 0.54	23.45 \pm 0.49
2	20	46.65 \pm 0.82	28.87 \pm 0.95
3	40	63.94 \pm 0.74	42.67 \pm 0.17
4	60	72.10 \pm 0.25	43.05 \pm 0.45
5	80	78.23 \pm 0.93	41.87 \pm 0.58
6	100	89.91 \pm 0.37	46.31 \pm 0.37
IC 50		16.70	94.80

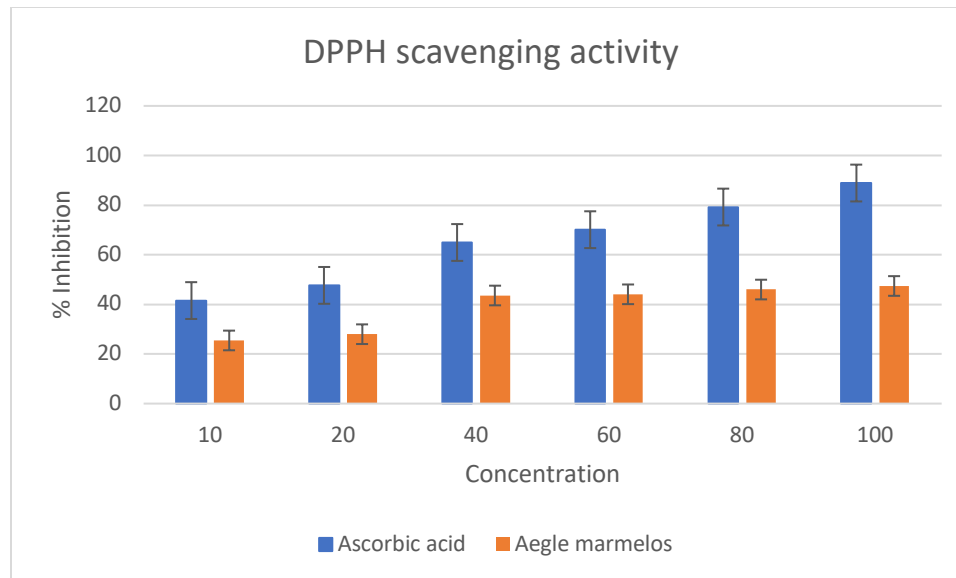


Figure 4.1. DPPH radical scavenging assay of ascorbic acid and *Aegle Marmelos* extract

4.1.2. Reducing Power

The reducing power assay is used to test the reducing capability of standardised extract AM and PM to convert the potassium ferricyanide (Fe^{3+}) complex to form potassium ferrocyanide (Fe^{2+}). The potassium ferrocyanide will then react with ferric chloride to form ferrous complex which can absorb maximally at 700nm. The reducing power of AM and Ascorbic acid were showed in table 4.2 and figure 4.2. Figure 4.2 showed pattern of increment in reducing power with increase in concentration of AM, PM and Ascorbic acid. Higher absorbance of the reaction mixture indicates higher reductive potential.

Table 4.2: Reducing power of AM and Ascorbic acid

S. No.	Concentration ($\mu\text{g/ml}$)	Reducing Power (absorbance)*	
		Ascorbic acid	<i>Aegle marmelos</i>
1	10	1.04 \pm 1.27	0.63 \pm 0.62
2	20	1.45 \pm 1.53	1.38 \pm 0.53
3	40	2.12 \pm 1.37	2.21 \pm 0.82
4	60	2.85 \pm 1.72	2.42 \pm 0.33
5	80	3.10 \pm 0.72	3.11 \pm 0.26
6	100	3.71 \pm 1.47	3.66 \pm 0.48

*All values are mean \pm SD (n=3)

4.2 Pre and Post Compression Parameter:

Pre and post compression studies of each trial batch showed the characteristics of granules and compressed tablet. Table 4.3 shows the pre-compression trials results and Table 4.4 show the post compression trials results.

Table 4.3: Results of Pre-compression studies of Polyherbal formulation

Pre formulation parameters	F1	F2	F3	F4	F5	F6
Bulk density (g/cm ³)	0.56±0.23	0.53±0.62	0.49±0.53	0.48±0.74	0.46±0.72	0.45±0.41
Tapped density (g/cm ³)	0.69±0.63	0.65±0.32	0.56±0.71	0.58±0.37	0.54±0.73	0.56±0.31
Angle of Repose (degree)	39±0.63	42±0.82	35±0.27	32±0.11	33±0.52	29±0.53
Compressibility (%)	18.84±0.45	18.46±0.4	12.5±0.57	17.24±0.3	14.81±0.19	19.64±0.4
Hausner's ratio	1.23±0.71	1.22±0.36	0.07±0.21	0.1±0.51	0.08±0.37	0.11±0.61

4.4 Evaluation Parameters of Formulation containing Methanolic Fractions:-

Evaluation of formulations containing methanolic fractions was done by various methods. The data represent in table 3.15.

Formulation F-1 showed hardness characteristics (4.83±1.33). Formulation friability (0.93±0.47) stated the tablets were structurally stable. Because the tablet average weight was 482.25±0.83, the weight variation is ±5%. The weight variance test was therefore passed by the entire developed tablet. Disintegration time of was 21.33±0.73minute.

Formulation F-2 showed hardness characteristics (4.93±1.36). Formulation friability (0.85±0.83) stated the tablets were structurally stable. Because the tablet average weight was 492.62±0.51, the weight variation is ±5%. The weight variance test was therefore passed by the entire developed tablet. Disintegration time of was 18.37±0.68minute.

Formulation F-3 showed hardness characteristics (5.06±0.89). Formulation friability (0.86±0.83) stated the tablets were structurally stable. Because the tablet average weight was 492.62±0.51, the weight variation is ±5%. The weight variance test was therefore passed by the entire developed tablet. Disintegration time of was 23.16±0.47minute.

Formulation F-4 showed hardness characteristics (5.11±1.25). Formulation friability (0.83±0.54) stated the tablets were structurally stable. Because the tablet average weight was 490.39±0.72, the weight variation is ±5%. The weight variance test was therefore passed by the entire developed tablet. Disintegration time of was 24.27±0.28minute.

Formulation F-5 showed hardness characteristics (4.91±1.29). Formulation friability (0.91±0.64) stated the tablets were structurally stable. Because the tablet average weight was 482.62±0.54, the

weight variation is $\pm 5\%$. The weight variance test was therefore passed by the entire developed tablet. Disintegration time of was 21.62 ± 0.58 minute.

Formulation F-6 showed hardness characteristics (5.03 ± 1.33). Formulation friability (0.89 ± 0.58) stated the tablets were structurally stable. Because the tablet average weight was 491.29 ± 0.61 , the weight variation is $\pm 5\%$. The weight variance test was therefore passed by the entire developed tablet. Disintegration time of was 23.11 ± 0.18 minute.

Table 4.4: Results of Post-compression studies of Polyherbal Formulation

Formulation	Average weight (mg)	Hardness (Kg/cm ²)	Thickness (mm)	% Friability	Disintegration Time (min)
F1	482.25 \pm 0.83	4.83 \pm 1.33	6.14 \pm 0.46	0.93 \pm 0.47	21.33 \pm 0.73
F2	492.62 \pm 0.51	4.93 \pm 1.36	6.11 \pm 0.83	0.85 \pm 0.83	18.37 \pm 0.68
F3	473.18 \pm 0.17	5.06 \pm 0.89	6.09 \pm 0.47	0.86 \pm 0.83	23.16 \pm 0.47
F4	490.39\pm0.72	5.11\pm1.25	6.18\pm0.63	0.83\pm0.54	24.27\pm0.28
F5	482.62 \pm 0.54	4.91 \pm 1.29	6.10 \pm 0.84	0.91 \pm 0.64	21.62 \pm 0.58
F6	491.29 \pm 0.61	5.03 \pm 1.33	6.11 \pm 0.46	0.89 \pm 0.58	23.11 \pm 0.18

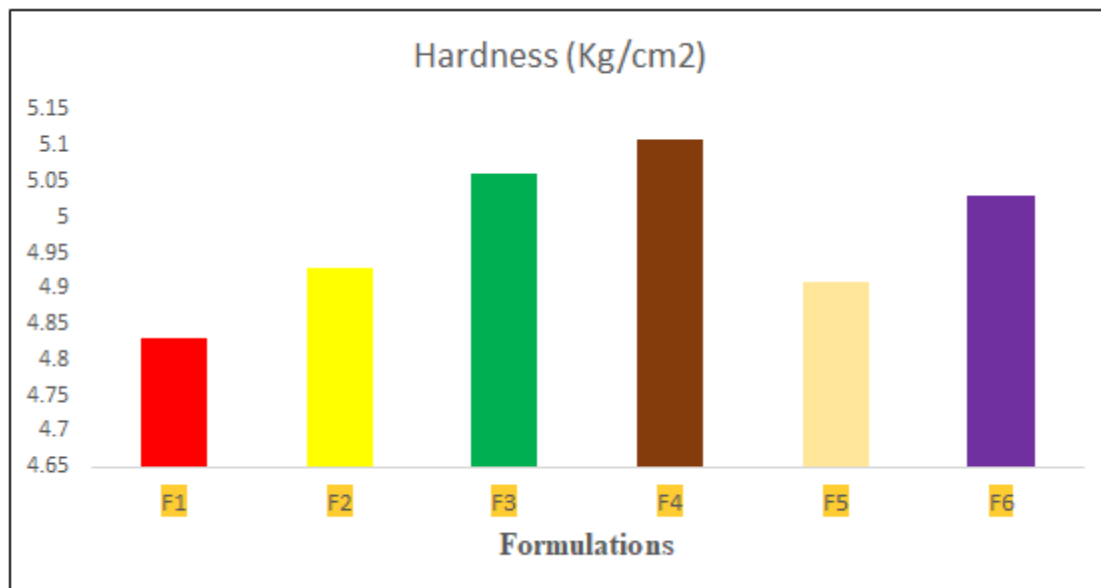


Fig 4.2- Comparative graph of Hardness of different formulation

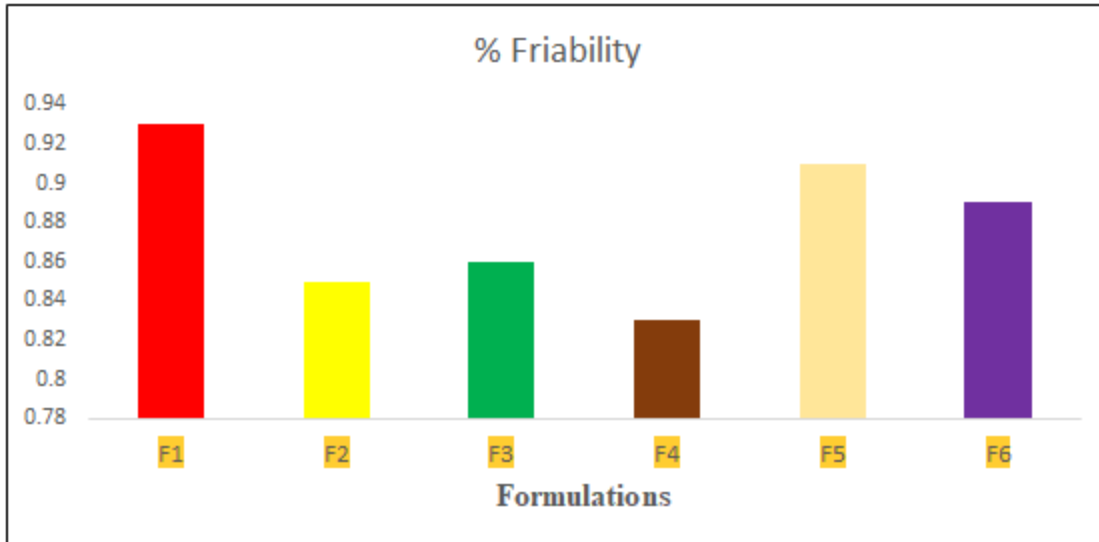


Fig 4.3- Comparative graph of Friability of different formulation

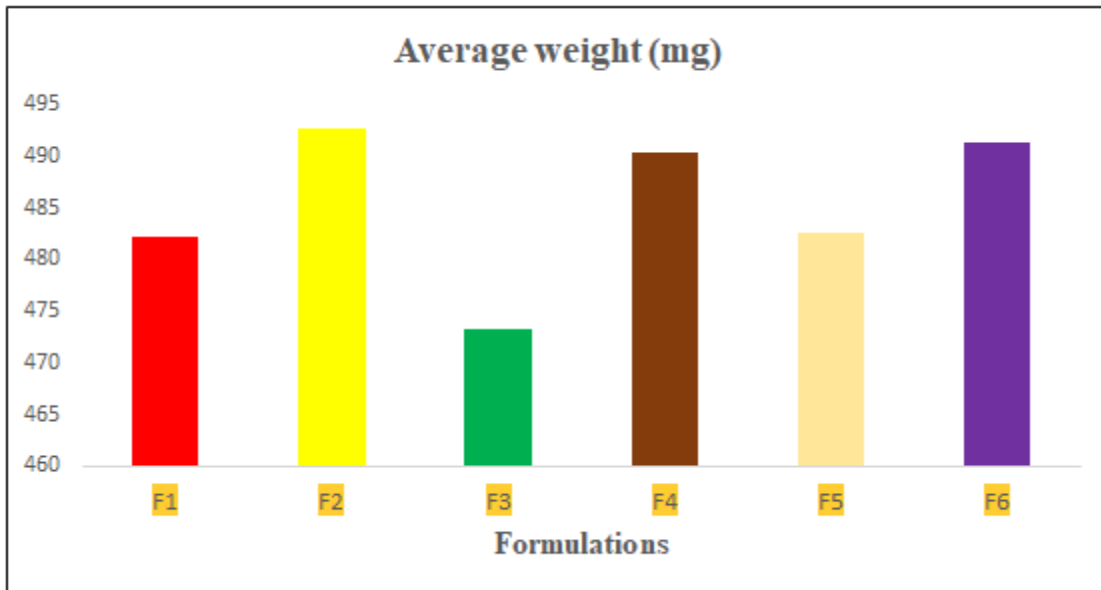


Fig 4.4- Comparative graph of Weight variation of different formulation

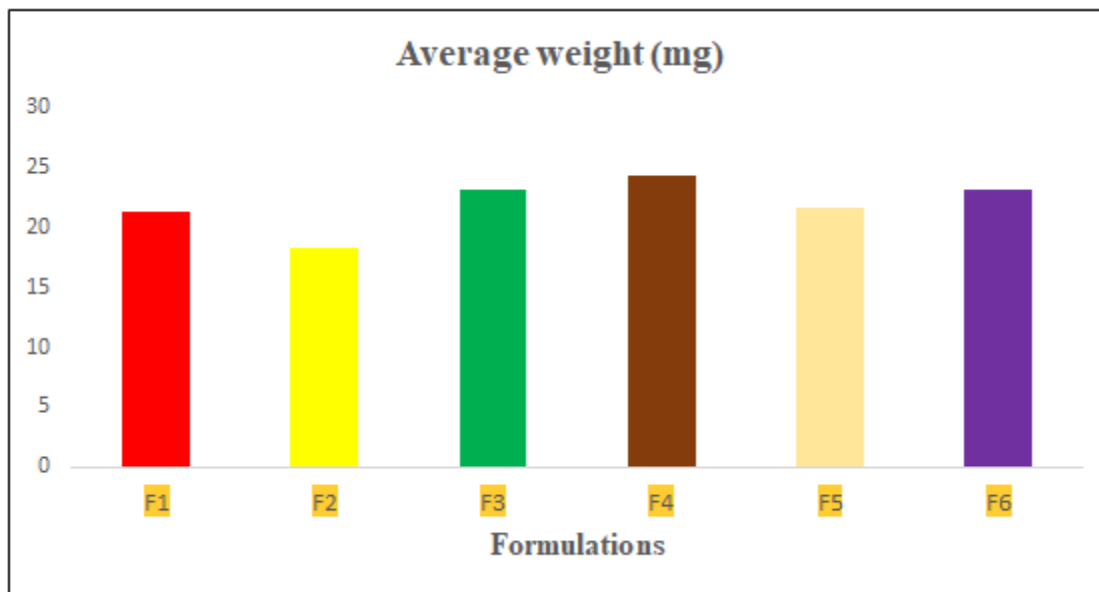


Fig 4.5- Comparative graph of Average Weight of different formulation

Conclusion:

Plants and other natural products have been in use since the ages for health and maintenance of life. The Vedic literature and ancient Indian scripture, gives the reference of numbers plants for prevention and cure for different diseases. Invitro antioxidant activity (DPPH free radical scavenging activity) was performed on selected extract of *Pedaliu murex* and *Aegle Marmelos*. IC₅₀ were calculated from % inhibition. IC₅₀ were found 94.80. The reducing power of *Aegle Marmelos* and Ascorbic acid were showed in table 4.2. Table 4.1 showed pattern of increment in reducing power with increase in concentration of AM and Ascorbic acid. Higher absorbance of the reaction mixture indicates higher reductive potential.

The reducing power assay is used to test the reducing capability of standardised extract AM and PM to convert the potassium ferricyanide (Fe^{3+}) complex to form potassium ferrocyanide (Fe^{2+}). The potassium ferrocyanide will then react with ferric chloride to form ferrous complex which can absorb maximally at 700nm. The reducing power of AM and Ascorbic acid were showed in table 4.2. Figure 4.2 showed pattern of increment in reducing power with increase in concentration of AM, PM and Ascorbic acid. Higher absorbance of the reaction mixture indicates higher reductive potential.

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