DETERMINATION OF IN-VITRO ANTIOXIDENT ACTIVITY OF ACTIVE FRACTION FROM AEGLE MARMELOS AND PEDALIUM MUREX LEAVES EXTRACT

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Abstract:
Aegle marmelos leaves and *Pedalium murex* has been used as a remedy for gastrointestinal infections, antidiarrheal, anti-inflammatory, anticancer, antidiabetic and antioxidant activity of human beings. Extraction was obtained using ethanol 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 nine fraction of *Aegle Marmelos* and *Pedalium murex* leaf extract was determined by using spectrophotometric method. The all fraction obtained from extract were based on solvent concentration for nine fractions. The total antioxidant capacity of the fractions was calculated in mg/ml of ascorbic acid with range between 48-132 mg/ml for *Aegle marmelos* and 43.3-116.3 mg/ml for *Pedalium murex*, total flavonoid content of the fractions was expressed of quercetin equivalent (QE) ranging between 47.5 – 83.5 mg/ml for *Aegle marmelos* and 112.6-146.3mg/ml for *Pedalium murex*, and total phenolic content of the fractions were expressed of Gallic acid equivalent (GAE) ranging between 108.2 – 181.5 *Aegle marmelos* and 152.6-211.5 for *Pedalium murex*. All the fractions, it was observed that methanol water fraction was having better antioxidant property.

Keywords: Active fraction, *Aegle marmelos, Pedalium murex, in-vitro* antioxidant activity, Fractionation.

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INTRODUCTION: -

[1]Human bodies possess enzymatic and non-enzymatic antioxidative mechanisms which minimize the generation of reactive oxygen species, responsible for many degenerative diseases including diabetes. [2] The disease is rapidly increasing worldwide and affecting all parts of the world. Due to deficiency of the insulin people suffering from diabetes have high blood glucose level. [3] However, many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects. [4] These limitations have largely prompted the exploration of management strategies involving the use of medicinal plants reported to be cost-effective antidiabetic agents with fewer reported side effects. [5] In countries like India, it is useful to employ a number of indigenous plant medicines due to the relatively high cost of allopathic medicines. [6]

There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience, and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Even the World Health Organization approves the use of plant drugs for different diseases including diabetes mellitus. [7] Plants are good source of drugs and majority of drugs are derived directly or indirectly from them. The ethnobotanical information reported about 800 plants may possess antidiabetic potentials. [8]

It is indigenous to India and is used in folk medicines.[9]
The Ayurvedic practitioners use almost all of their parts but the greatest medicinal value ascribed to its fruits.
Oxidative stress is produced during normal metabolic process in the body as well as induced induced by a variety of environmental and chemi-cal 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 act ivity of *A. marmelos* and *Pedalium murex* fruit pulp has been reported so far.[10-12]

2. MATERIAL AND METHODS:

2.1. Collection of plants leaves:

The leaves of *Aegle marmelos* and *Pedalium murex* was collected from local regions of Madhya Pradesh, 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* and *Pedalium murex* was carried out by soxhlet extraction method. The solvent ethanol (250ml) was placed in a round bottom flask isolated with a Soxhlet extractor and condenser (Figure 6.1). The crushed leaves(500gm) were placed in a thimble and the thimble was inserted into a Soxhlet extractor. The side arm was covered with glass wool. The solvent was heated using a heating source (isojacket), travels through the device to the condenser and begins to evaporate. The condensate then drops into the reservoir with the sleeve. When the solvent reaches the siphon, it flows back into the flask and the cycle resumes. After completion of extraction, the solvent was removed by distillation. [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, the lower part of the glass column was stocked with glass wool with the aid of glass rod. 75g of silica gel (G60-200 mesh size) was dissolved in 180 ml of absolute chloroform to make the slurry.

The chromatographic column (30mm diameter by 40 cm height) was packed with silica gel and was allowed free flow of the solvent into a conical flask below. The set up was seen to be in order when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed 24 h to stabilize after which, the clear solvent at the top of the silica gel was allowed to drain down 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. Elution of the extract was done with solvent system of gradually increasing polarity, beginning from chloroform, ethyl acetate, methanol and finally water. The

following ratio of solvent combination was sequentially used in the elution protocol:
i) Chloroform: ethyl acetate 90:10, 50:50, 25:75.
ii) Ethyl acetate: methanol 50:50, 90:10,25:75
iii) Methanol: water 90:10,50:50,25:75
A measured volume (500 ml) of each solvent combination was poured into the column each time using separator funnel. The eluted fractions were collected in aliquots of 500 ml in fraction collection tubes.

Determination of Total Flavonoid Content (TFC):
Determination of total flavonoids content was based on aluminium chloride method [15,16].10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared 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.

Estimation of Total phenol content estimation (TPC):
The total phenol content of the extract was determined by the modified folin-ciocalteu method [17]. Total polyphenol component was estimated colorimetrically at 765 nm as described by Lachman et al., using Follin-Ciocalteu reagent and expressed as gallic acid equivalent (GAE). The reactions were conducted in triplicates and absorbance of the sample was measured against the reagent blank at 765nm.

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 method described by Singleton et al.,[18]. The absorbance was measured at 517nm in triplicate for each fraction. Total antioxidant capacity (TAC) was calculated as mg/ml of ascorbic acid equivalent using the regression equation from calibration curve.

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 and table 2.

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 (90:10)	47.5	108.2	48
2	Chloro.: Eth. Acetate (50:50)	58.3	96.3	35
3	Chloro.: Eth. Acetate (25:75)	76.5	116.5	47
3	Eth. Accet. :Etha. (90:10)	78.2	128.3	53
5	Etha. : Meth (50:50)	62.4	119.5	58
6	Etha. : Meth. (25:75)	71.4	123.2	48
7	Meth: water (90:10)	78.2	133.4	42
8	Meth.: water (50:50)	80.5	160.5	75
9	Meth.: water (25:75)	**83.5**	**181.5**	**132**

Table no 2: Total flavonoid content, Total polyphenol content and total antioxidant capacity of *Pedalium murex*.

Fractions	Solvent System	TFC (mg/ml)	TPC (mg/ml)	TAC (mg/ml)
1	Chloro.: Eth. Acetate (90:10)	112.6	152.6	43.3
2	Chloro.: Eth. Acetate (50:50)	105.4	141.4	34.3
3	Chloro.: Eth. Acetate (25:75)	118.3	147.2	59.3
3	Eth. Accet. :Metha. (90:10)	132.8	152.1	63.2
5	Etha. : Meth (50:50)	128.5	182.4	66.4
6	Etha. : Meth. (25:75)	123.7	161.7	73.1
7	Meth: water (90:10)	141.4	169.6	81.4
8	Meth.: water (50:50)	143.2	180.5	101.2
9	**Meth.: water (25:75)**	**146.3**	**211.5**	**116.3**

The correleation between TFC vs TPC, TPC vs TAC, TAC vs TFC for *Aegle marmelos* ethanolic 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.

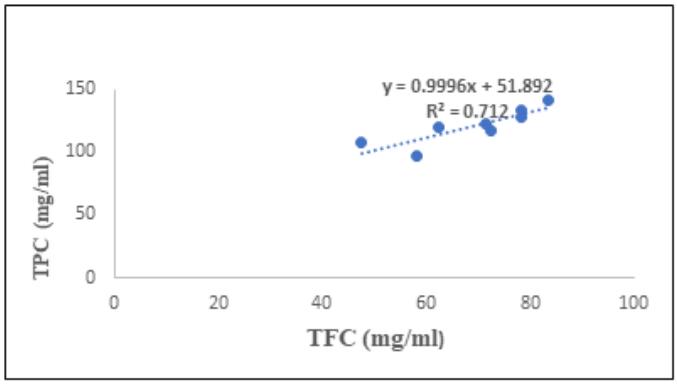

Figure 1: Correlation between total flavonoid content and total polyphenol content of fraction obtain from ethanolic extract of *Aegle marmelos* leaf extract.

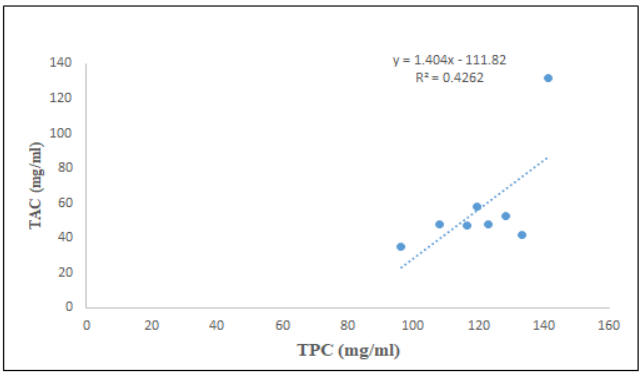

Figure 2: Correlation between total polyphenol content and total antioxidant capacity of fraction obtain from ethanolic extract of *Aegle marmelos* leaf extract.

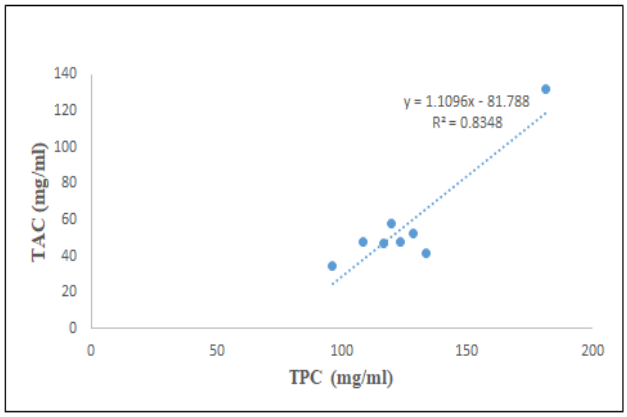

Figure 3: Correlation between total antioxidant capacity and total flavonoid content of fraction obtain from ethanolic extract of *Aegle marmelos* leaf extract.

The correleation between TFC vs TPC, TPC vs TAC, TAC vs TFC for *Pedalium murex*

From the results of above data that all the correlations were positive correlation but flavonoid content is the better (Figure 6) correlation with Total antioxidant capacity (TAC). And also positive correlation was observed in other parameter show in figure 4 and figure 5.

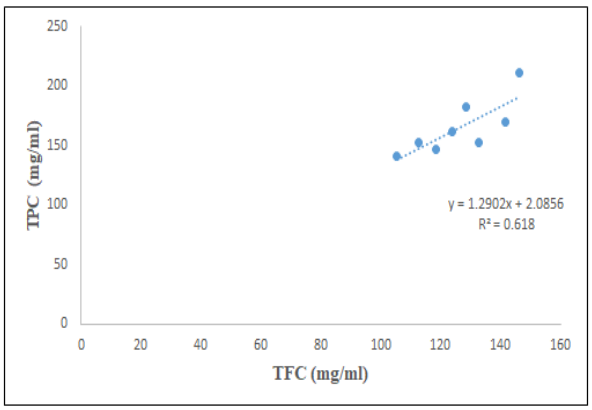

Figure 4: Correlation between total flavonoid content and total polyphenol content of fraction obtain from ethanolic extract of *Pedalium murex* leaf extract.

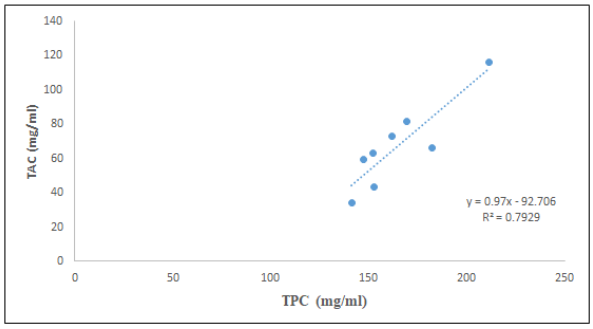

Figure 5: Correlation between total polyphenol content and total antioxidant capacity of fraction obtain from ethanolic extract of *Pedalium murex* leaf extract.

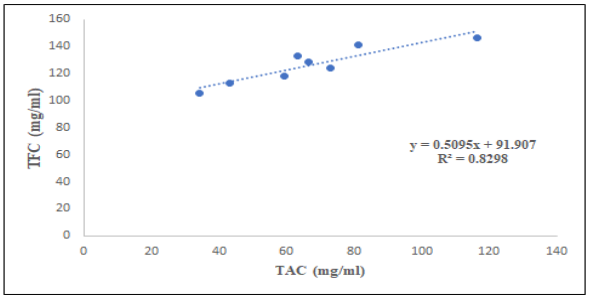

Figure 6: Correlation between total antioxidant capacity and total flavonoid content of fraction obtain from ethanolic extract of *Pedalium murex* leaf extract.

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) and *Pedalium murex* 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) for *Aegle marmelos* and (R^2 =0.792) for *Pedalium murex* 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* and *Pedalium murex* leaves depend on the polarity of the eluting solvent.

The total antioxidant of *Aegle marmelos* and *Pedalium murex* leaves ranged from 47.5-83.5 mg/ml and 112.6-146.3 Quercetin equivalent and total phenol capacity ranged from 108.2-181.5 mg/ml and 152.6-211.5 and GAE (table 1) and (table 2) from this report the total flavonoid capacity of Ethanol extract of *Aegle marmelos* and *Pedalium murex* may be responsible for Antioxidant activity since a strong positive correlation (R^2= 0.8348) and (R^2= 0.8298) is observed with the total antioxidant capacity as shown in figure 3 and 6.

In the current study there was a strong relationship between Total antioxidant and total flavonoid content of *Aegle marmelos* and *Pedalium murex* leaves. Therefore, it can be said that the total antioxidant capacity of a fraction is majorly dependent on their flavonoids content.

CONCLUSION:

The results of this study showed that the highest antioxidant activity and Total phenol capacity and total flavonoids content respectively of *Aegle marmelos* and *Pedalium murex* leaves ethanol extract was exhibited by the methanol water fraction. Hence, there was strong positive correlation between the TAC and the TPC, indicating that the flavonoids content of the extracts are to a larger extent responsible for the elicited antioxidant effects of the extract.

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