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PHYTOCHEMICAL ANALYSES AND ANTIOXIDANT ACTIVITY OF AERVA JAVANICA (BURM. F.) JUSS. Ex SCHULT

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ABSTRACT

This paper tuckels the phytochemical analysis of the *Aerva javanica* which has revealed that the plant contains various chemicals as flavonoids, tannins, alkaloids, saponins and phenols. This study was carried out to evaluate the amount of flavonoids and phenols stored inside of the pedicel and petals using the following solvents chloroform, ethyl acetate and n-butanol which resulted in six extracts. Highest phenolic content (100.82±1.47 mg GAE /g extract) for ethyl acetate stems extracts, while highest flavonoid content (101.46±0.09 mg QE /g extract) for chloroform flowers extracts. The antioxidant activity was investigated using the DPPH profound rummaging activity and phosphomolybdenum tests. It also includes how the combination of both the total phenolic contents and total flavonoids achieved the major results. Chloroform petals extracts had the highest chemical group scavenging activity byIC50 of 0.1035±0.03mg/ml. In addition, the phosphomolybdenum has achieved the highest result (67.72 mM) for n-butanol compared to the other solvents. The chemicals extracted from the *Aerva javanica* (Burm. f.) portray a postive antioxidant activity

Keywords: Aerva javanica plant, stem extracts and flower extracts, Total phenols content, Total flavonoid content, chemical group scavenging activity, phosphomolybdenum, correlation coefficient.

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1. INTRODUCTION

The *Aerva javanica* plant belongs to the Amaranthaceae family. It is originally located in a wide part of Africa and some Asian countries. It is considered widespreaded, and is known for its high construct calculated as 1.5 m high and for the dense hairs covering both the leaves and the pedicel. The Aerva javanica plant is much-branched with round stems and it has numerous leaves, while the petals are whitish small.

In traditional medicine, herbal medicines take a huge part of the world's primary population as 80% in the developed countries [1-3]. *The Aerva javanica* plant helps to reduce swellings and treat the disorder of the urinary system. The plant assits domestic animals suffering from ulceration by applying its powder over the externall surface. Aerva javanica roots have plenty of usages; in Ethiopia for instance, they are used to administer the goat's ophthalmic infection [4], they are also used in the treatment of swelling, rheumatism, inflammation, headache and kidney problems. Moreover, the seeds are rich with medical properties versus rheumatism [5]. The phytochemical analyses are necessary in the investigations of roles of the herb in medicine fields. Several phytochemical scanning has been published, the major chemical substances of interest in these surveys has been the alkaloids and steroidal, saponins. In addition to the largest natural existing phytochemical groups as phenols and flavonoids, which have become the topic of medical research and they consist of many beneficial characteristics including the antioxidant activity. The present study mainly focuses on the investigation of the oxidation inhibitor activity and the phenolic and flavonoid contents of different essences stored in the *Aerva javanica* (Burm. f.) plant.

2. PLANT PREPARATION AND EXTRACTION

The plant material was collected from the Tamanrasset region in southern Algeria in the late of November 2016. The collected petals, pedicels and root samples that are during the dryer periods were rich in secondary metabolites. After disconnecting the Aerva janavica into three parts and storing them away from heat and humidity until used. In February, the three parts was crushed. 2 grams of each sample were macerated in 20 ml (20% pure water and 80% methanol) for 24 hours, then filtered to be used in the intended tests.

Fig.1. (A) *The Aerva javanica* plant, (B)petals and pedicels of *Aerva javanica*

2.1. Phytochemical analysis

The phytochemical analysis of the raw methanolic extracts from pedicels, petals and roots were tested in order to discover the existence of different phytochemicals. The qualitative method classifies the results into two categories; the (+) and the (-) refer to the presence and the absence of phytochemicals

Testing for alkaloids

In this test 3 ml of dissolved extract was stirred and added into 1% fluid hydrochloric acid (1 mL) and heated on a steam bath. 1 mL was treated by Mayer's reagent (3 drops) with the emergence of yellow- white precipitate, while another portion was similarly treated by Dragendorff's reagent with the emergence of the orange red precipitated. Turbidity with these reagents was considered as evidence of the alkaloids existence.

Testing for Coumarin

In the test tube, 3 ml (10%) of NaOH was blended with aqueous extract (2 ml). Test tube was left couple minutes on a steam bath. The color changing into yellow signifies the existence of coumarins.

Testing for steroids

Using the Salkowski test, preparing a mixture of 2 ml of chloroform and 2 ml concentrated H_2SO_4 combined with 5 ml of aqueous extract. As a result, a red color appeared in the bottom layer of chloroform; this positive result of the test confirms the existence of Sterols.

Testing for Terpenoids

Using the previous test (Salkowski), mixing 5 ml of the extract aqueous with 2 ml chloroform then adding carefully concentrated H_2SO_4 (3ml) to formulate layer, this process results a

change of color to reddish brown a positive sign of the existence of terpenoid.

Testing for tannins

In the ferric chloride test, 2 ml the aqueous extract was poured into a test tube with 2 ml of (2 %) $FeCl_3$ solution, (50 ml) of pure water and 1 g of $FeCl_3$). The emergence of dark blue color signifies the existence of tannins.

Testing for Phenol

By the ferric chloride test: aqueous extracts were treated with4 drops of alcoholic $FeCl_3$ solution. Bluish black color formation confirmes the existence of phenol.

Testing for flavonoids

Using Shinoda Test, adding 2 ml of aqueous extract to intence hydrochloric acid (1ml) and 5 small pieces of magnesium. The appearance of reddish purple color indicates the existence of the flavonoids.

Testing for saponins

In the foam test, whipping in a test tube 2 ml of the aqueous extract and (5 ml) of pure water separately for 15 minutes, the formation of foam indicates the existence of saponins.

2.2. Extraction of phenolic composites

As for the aqueous extract to be secured, 100g of the plant sections (petals and pedicels) would be macerated in 1000 ml of 80% methanol and 20% pure water. The mixture was stirred by a magnetic stirrer for 48 hours at ambient temperature (27-28°C). The extracts were separated by filter paper. The solvent was removed by a rotary evaporator at 40 °C. Undergoing 50 ml of aqueous phase in lyophilization and then storing it at -20 °C. The rest of the mixture (7g flowers powder, 9g stems powder) will be dissolved in 90 ml pure water with dilapidation by petroleum ether until the total absence of the color, hence the organic and the aqueous stages are obtained. The aqueous stage is decanted with chloroform to given an organic phase (2) (chloroform extract A1, A'1 of flowers and stems) and an aqueous phase (2). The aqueous phase (2) is decanted with ethyl ester to obtain an aqueous phase (3) and organic phase (3) which is represented by ethyl acetate extract A2, A'2 of flowers and stems respectively. The aqueous stage (3) undergoes a final extraction with n-butanol to obtain an organic phase A3, A'3 and aqueous phase. Finally, six samples are obtained as presented in the

the diagram of figure 2.

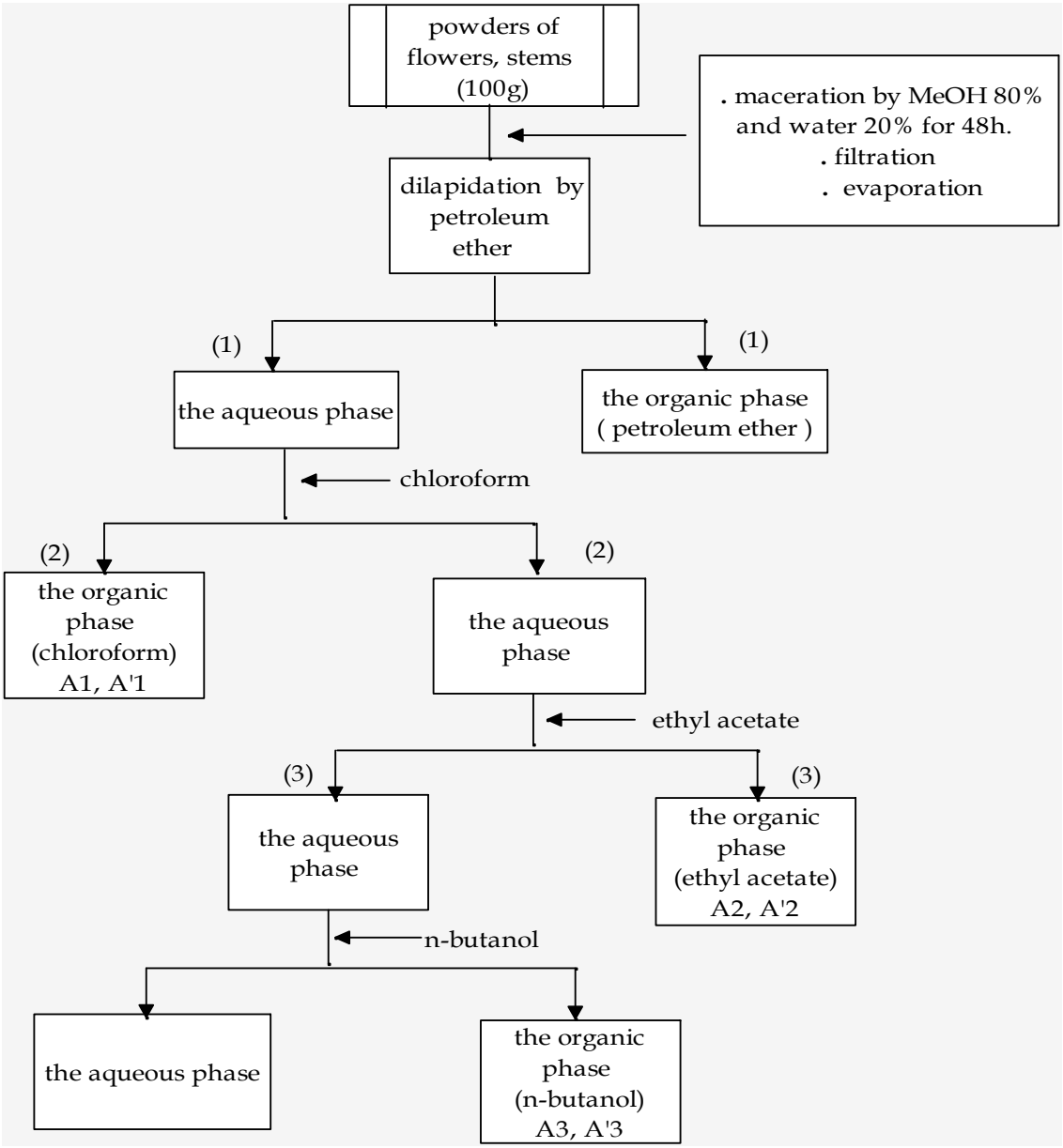

Fig.2. The steps of the phenolic compound extraction by using non-chemical solvents

2.3. Chemical composition of plant essences

2.3.1 Phenols content determination

The total phenolic amount in petals and pedicels of The Aerva javanica was determined by using the Folin-Ciocalteu colorimetric method. Different concentration of gallic acid was prepared to draw the measurement curved line. Preparing the mixture requires dissolving

0.03g of gallic acid in 100 mL pure water. The concentrations prepared through dilution were 0.03, 0.06, 0.12, 0.15, 0.18, 0.21, 0.24, 0.27 and 0.3 g/L of gallic acid. In addition, Folin-Ciocalteu's phenol reagent was diluted 1:1 in distilled water before use. The progression curved line was prepared by mixing 0.5 mL of different concentration of diluted gallic acid, 1.5 mL of (10%) Folin-Ciocalteu reagent and 3 mL of dissolved sodium carbonate (75 g/L); After thirty minutes the devouringness was measured to result as 765 nm. 0.5 mL extracts were mixed separately, with the previous reagents as did in construction of calibration curve and kept in the dark for half an hour at ordinary temperature. The absorption was calculated as765 nm using a UV/Visible spectrophotometer against blank, which returned to as a reference. All the measurements were caculated in three copies and expressed in mg/g to extract the weight of the gallic acid equivalent (GAE). The results were determined using the equation (1).

$$TPC = \frac{A \cdot V.F}{K.P} \qquad (1)$$

Where, A : Absorption at 760 nm; K: Declining of the curved line of gallic acid

F: Extension factor for extract; V: Volume of extract in ml ;

P: Weight of the extract, expressed in grams.

2.3.2 Flavonoid content determination

The procedure of the aluminum chloride colorimetric is used in each excerption as to determine the total amount of flavonoid [6]. In order to detect its amount, quercetin was used to create the progression curved line. The quercitroside solution preparation demands dissolving 5.0 mg quercetin in 1.0 mL CH_3OH, after that the standard quercetin liquiforms formation included a serial dilutions using methanol (0.5–5 mg/mL). An amount of 0.5 mL diluted standard quercetin solution or extract was independently blended with 0.5 mL of 2% ($AlCl_3$) aluminum chloride. After blending, the solution was held for 30 min at room temperature. The absorption of the interacting mixtures was measured against blank at λ=430 nm wavelength

using pectrophotometer. The samples of extracts were prepared in three copies for analysis for calculating the flavonoid amount using formula (2):

$$TFC = \frac{\grave{A} \cdot \grave{V}.\grave{F}}{\grave{K}.\grave{P}} \quad (2)$$

Where, $\grave{A}$: Absorption at 430 nm; $\grave{K}$: Curve slope of quercetin

$\grave{F}$: Extension factor for extract; $\grave{V}$: Volume of extract in ml;

$\grave{P}$: Weight of derivings in grams.

2.4 Antioxidant activity assay

2.4.1 DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

In this test, the anti oxidizing activity of the extract was measured by the use of the DPPH method of Brand-William [7] with several changes and which presents radical scavenging ability of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals. In this study, Vitamin C was utilized as a reference; in a test tube the following chemicals were combined, 10µl of each extract with varying concentrations and (190 µl) of a 0.250 mmol/l DPPH˙ ethanol solution. The resulted combination was incubated in the dark condition for half an hour at ambient temperature. The absorption value deacreased to 517 nm using (UV-VIS) spectrophotometer. Whereas the IC50 value of the concentration of compound was calculated when the inhibition degree matched 50%. The results computed using the following formula (3):

$$\text{DPPH inhibition (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (3)$$

Where: $A_{control}$ refers to the absorption of the DPPH solution without extract ,and A_{sample} refers to the absorption of sample combined with the DPPH solution.

2.4.2 Total Antioxidant Capacity (TAC) by the use of the Phosphomolybdenum test

The overall capacity of the samples anti oxidizing (TAC) were evaluated based on Prieto method [8]. In summary, Vitamin C standed as a positive standard reference. The combination results by mixing 0.3 ml of each extract solution with 3 ml of molybdate reagent solution into test tube. The Reagent composition involves mixing 28 mM sodium dihydrogen phosphate (NaH_2PO_4) and 4 mM ammonium heptamolybdate in 0.6 M sulfuric acid (H_2SO_4). This procedure indicates covering the test tube aluminum sheet so that it blockes the light then put in a steam bath at 95 °C for 90 minutes. After the solution chills absorption value

reached 695 nm against the blank using a UV spectrophotometer. Inhibitors capacity was stated as mM similar to ascorbic acid.

3. RESULTS AND DISCUSSION

The remedial features of the *Aerva javanica* plant might be own up to the of varied secondary metabolites. The present study was accomplished as to provide information on the phytochemical components found in the petals, pedicels and roots that are often utilized in traditional medicine to cure numerous conditons. The results of this analysis are presented Table 1.

Table 1: Qualitative analysis of the chemical components of the *Aerva javanica (*Burm. f.*)*

Phytochemicals names	**Part used**		
	Flowers	Stems	Roots
Alkaloids	-	-	++
Flavonoids	+++	++	-
Tannins	+	++	-
Phenols	++	+++	-
Saponins	+++	++	+
Triterpenoids	+	++	-
Steroids	+	+	-
Coumarins	+	+	-

In three parts. (+++) very strongly present, (++) moderate present, (+) poor Present, (-) absence.

The phytochemical substances such as phenolic compounds, flavonoids, steroids, saponins and tannins which have received increasing interest over the last decades given their curing chracteristics against most diseases. The examination of the the phytochemical elements reveals that the roots of the herb tested positive for alkaloids. While the presence of phenolics, flavonoids, terpenoids, saponins, tannins in petals and pedicels.

3.1. Content of polyphenols, flavonoids

It is possible to determine the amount of polyphenols in extracts of chloroform, ethyl acetate and n-butanol, in order to rate the potential anti oxidizing capacity of the extracts from petals and pedicels of the *Aerva javanica* (Burm. f.*).* The total phenols, and flavonoids were checked

for all examined extracts by spectrophotometric methods and the results are submitted in Table 2.

Table 2: Comparative surveys between the total flavonoid content and total phenolic content collected from extracts of *Aerva javanica*

Extrants		Total phenolic content (mg GAE /g extract).	Total flavonoid content (mg QE /g extract).
Flowers	Chloroform	99.79±0.08	101.46±0.09
	Ethyl acetate	14.84±0.10	32.20±0.13
	n-butanol	53.31±0.18	56.69±0.05
Stems	Chloroform	98.63±0.57	48.86±0.07
	Ethyl acetate	100.82±1.37	51.52±0.04
	n-butanol	22.99±0.24	25.70±0.09

The results were expressed as ±SD (n=3), GAE = gallic acid equivalent; QE = quercetin equivalent

The entire components of phenol were recorded ranging from 14.84 mg QE/g extract to 100.82mg QE/g extract through chloroform, ethyl acetate and n-butanol extract. Results indicated that ethyl acetate stem extracts had the highest phenolic content (100.82 mg GAE /g extract) followed by chloroform flower extracts (99.79 mg GAE/g). This result might be the reason of the complex structure of some phenolic components in the extract that are soluble in chloroform, ethyl acetate and n-butanol. Interestingly, chloroform. Petals extracts has the major value of flavonoid content, while ethyl acetate stem extracts have the major phenolic value as shown in the table 2. In addition to the full composes of phenol obtained in chloroform was higher than the results reported by Musbau Adewunmi Akanji in leaves extracts 30.72 ± 3.01 mg GAE/g (Ethanol),55.91 ± 2.75 mg GAE/g (Aqueous) and 78.15 ± 2.50 mg GAE/g in Hydroethanol for *Aerva lanata* [9], O. Raihan et al (108.91 mg GAE/g extract) for methanol extract taken out of the Aerva lanata parts which ere exposed to the air [10], B. mandal at al which reported a maximum of 127.84 mg GAE/g essence for Aerial fragments of *Aerva lanata* **(**Linn**.)** Juss. ex Schult[11]. Some studies have also shown entire phenolic composes values between 100 and 150 mg GAE/g, with the acetone and the

methanol of leaves, stems and flowers of *Thermopsis turcica* [12]. Generally, these values are higher than our results. The full flavonoid composes obtained was recorded ranging from 25.70mg QE/g to 101.46 mg QE/g of the different extracts, which was highest from 77.61 mg QE/g for *Aerva lanata* **(**Linn**.)** [11], 140.34 mg QE/g for *A. lanata* [10], 7.89 ± 0.11 to 17.67 ± 0.25 mg QE/g essence with aqueous, ethanol and hydroethanol [12].

Table 3: Values of IC_{50} to segments of the *Aerva javanica* plant

Plants part	Solvent	DPPH(IC_{50} (mg/ml))± SD
petals	Chloroform	0.1035±0.0378
	Ethyl acetate	0.2480±0.007
	n-butanol	0.1323±0.012
pedicels	Chloroform	0.1960±0.0156
	Ethyl acetate	0.1735±0.0163
	n-butanol	0.3010±0.025
	Ascorbic acid	0.07 ±0.0045

Values are refered to as mean ± SD (n = 3) of triplicates

A lower value of IC_{50} indicates a higher antioxidant activity, and hence the antioxidant properties measured as IC_{50} of the plant segments starting from the most active were in petals: (0.1035 ±0.0378 mg/ml) chloroform > n-butanol (0.1323 ±0.012l mg/m) > ethyl acetate (0.2480 ±0.007 mg/ml), while extracts of pedicels were ranked as: ethyl acetate (0.1735 ±0.0163 mg/ml) > chloroform (0.1960 ±0.0156 mg/ml) > n-butanol (0.3010 ±0.025 mg/ml). It is clear that the strongest DPPH activity was accessed in chloroform petals extracts, while the lowest activity was obtained in n-butanol pedicels extracts. The standard used was ascorbic acid with IC50 value at 0.07 ±0.0045mg/mL, where IC_{50} value for ascorbic acid depend on the assay used in the laboratory. Study this, we used correlation analysis, which showed positive bond between the inhibitors capacity IC_{50} with flavonoid components and phenolic content found that the correlation between IC50 and flavonoid content was R^2=0. 7687(flowers) and R^2=0.9948 (stems), while the correlation between IC_{50} and phenol content was R^2=0.8557 (flowers) and R^2=0.9801 (stems) as displayed in figure 3 and Figure 4. Furthermore, there is also a very high correlation between total flavonoid content and full contents of phenol (R^2= 0.9878 (flowers) and R^2=0.9952 (stems). This result indicates clearly

that the free-radical rummaging competence was substantial in the case of both flavonoids and phenols. Hence, It might be suggested that the combination of phenolic and flavonoids unites is a key factor of the radical scavenging activity.

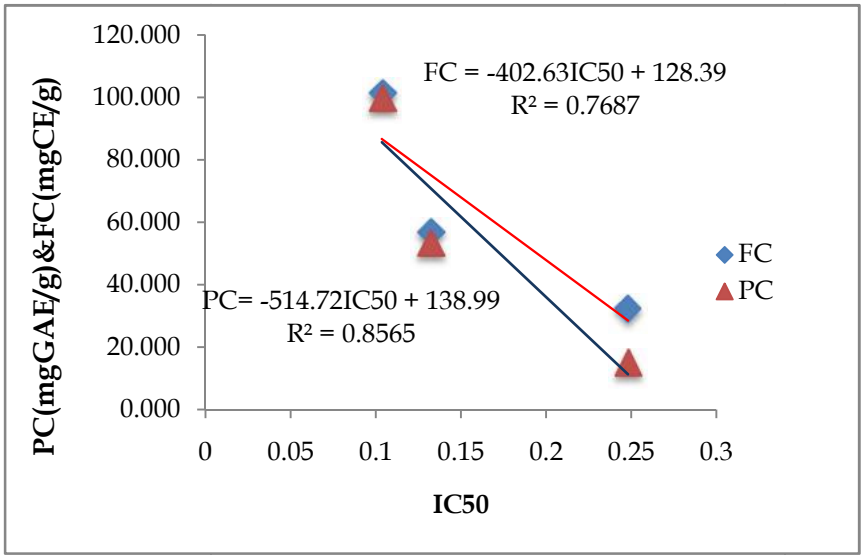

Fig.3. *The connection between phenolic and flavonoid content, (PC) and (FC) with anti oxidizing capacity (IC_{50}) in the company of chloroform, Ethyl acetate and n-butanol petals extracts*

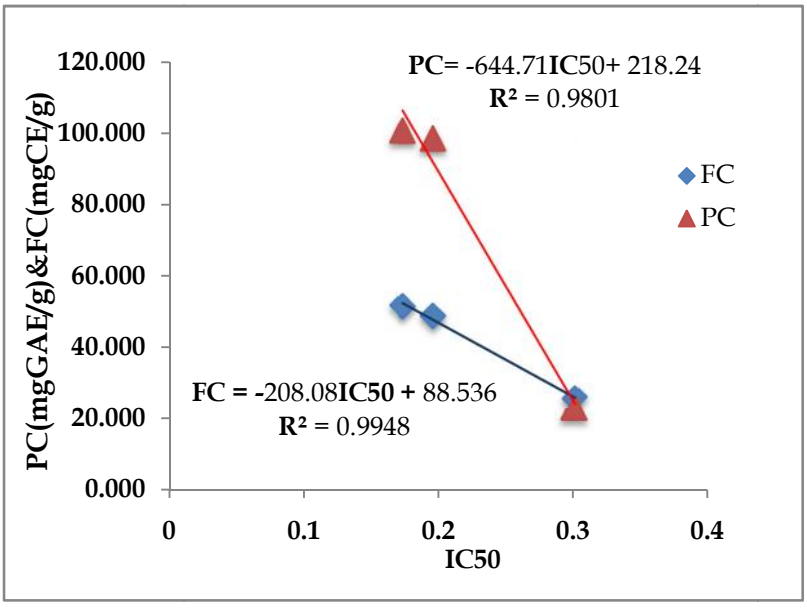

Fig.4. *The connection between phenolic (PC) and flavonoid content (FC) with antioxidant capacity (IC_{50}) in diluents of chloroform, ethyl acetate and n-butanol stems extracts.*

3.2.2 Phosphomolybdenum Test

The phosphomolybdate assay is quantitative, since the complete antioxidantive capacity is conveyed as ascorbic acid equivalents. The results confirmed that the inhibitors aptitude was directly related to the nature of the used extract and to the mechanism of the electronic transmission of Mo (IV) to Mo (V). The oxidant ability in the n-butanol extract was much higher activity potential in comparison with the two other extracts. In flowers part, value of n-butanol (67.72 ±011 mM) was the best antioxidant as demonstrated by the median value of phenolic composes (53.31 ±0.18 mg GAE /g) and flavonoid content (56.645 ±0.05 mg GAE /g). While in stems, the high value of n-butanol (41.389 ±0.050 mM) was with the lowest value in phenolic content (22.99 ±0.24 mg GAE /g) and flavonoid content (25.708 ±0.09 mg GAE /g) as listed in Figure (5,6). This study shows that n-butanol is the best milieu for reduction of Mo (VI) to Mo (V) on one hand, and on other hand many natural products can lead to a decline such as phenols and flavonoids. However, some studies indicated that flavonoid content related polyphenol stand as a relevant participant to the phosphomolybdate scavenging activity of medicinal plants [13, 14].

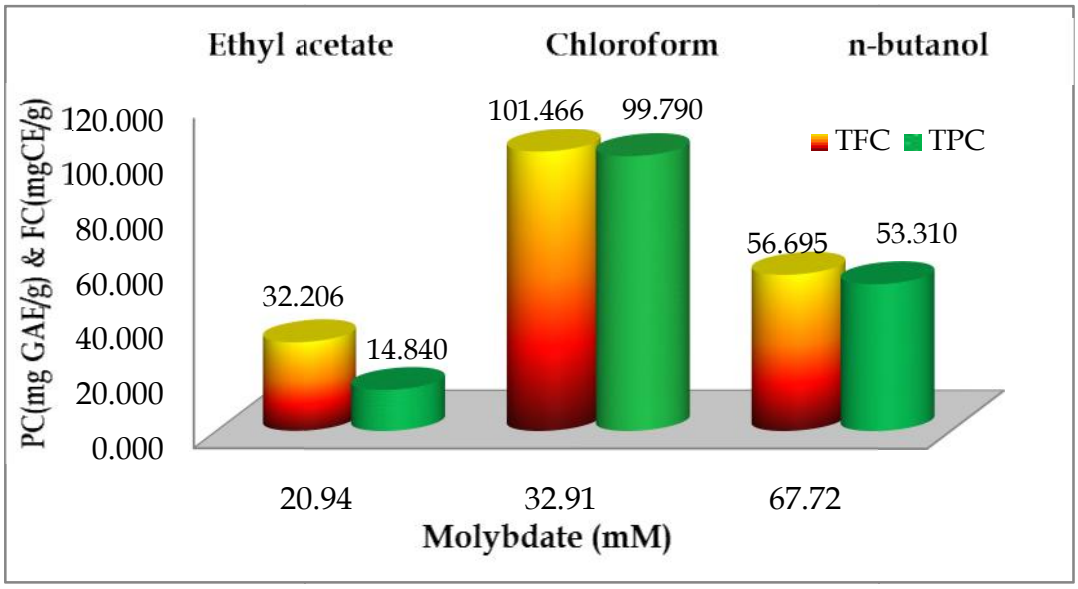

Fig.5. The full anti oxidant ability obtained through the phosphomolybdenum testwith phenolic and flavonoid composes according to solvent extracts of flowers

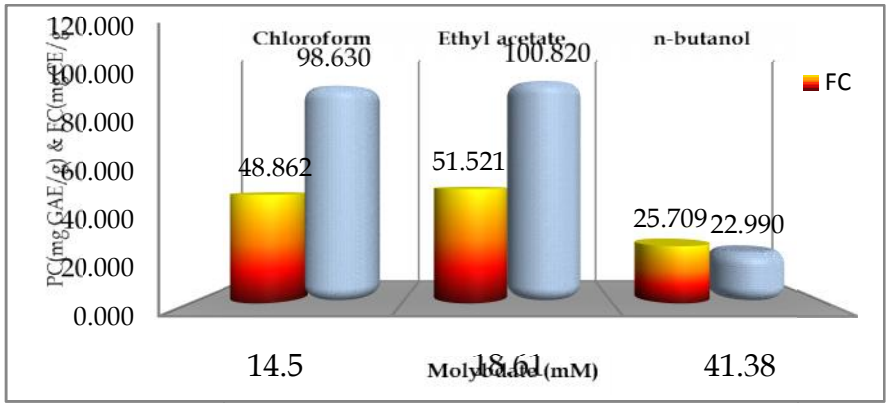

Fig.6. The full anti oxidant ability obtained by the phosphomolybdenum test with phenolic and flavonoid composes of different solvent extracts of stems

4. CONCLUSION

To sum up, the previous results revealed that *Aerva javanica* extracts exhibit well anti oxidizing potential. It was found that highest antioxidant capacity in the n-butanol extract. The herb extraction amount of total phenols, Flavonoid. All of which assist to the observed antioxidant activity. The root rummaging activity was impacted by the extract components and the solvent use, where the results gained from pedicels extracts were better than flowers extracts. In addition, total the flavonoid components and the phenolic were highly correlated with one another. Notwithstanding, we believe that it is essential to make further studies on the main active ingredients of the extracts and their action mechanism in this plant. Finally, *the Aerva javanica* plant can be an antioxidants strong, advantageous remedy for human kind.

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