Antioxidant Activity, Phenolic and Flavonoid Contents of Ephedra Alata in Southeast Algeria

Elyacout Chebouat[*] [1], Derradji Hadef [2], Imane Chebouat[3], Mohamed Lakhdar Belfar*[4]
[1] VPRS Laboratory, Chemistry Department, Faculty of mathematics and Matter sciences, University Kasdi Merbah, Ouargla 30000, Algeria.
[2] VPRS Laboratory, Chemistry Department, Faculty of mathematics and Matter sciences, University Kasdi Merbah, Ouargla 30000, Algeria.
[3] VPRS Laboratory, Chemistry Department, Faculty of mathematics and Matter sciences, University Kasdi Merbah, Ouargla 30000, Algeria.
[4] VPRS Laboratory, Chemistry Department, Faculty of mathematics and Matter sciences, University Kasdi Merbah, Ouargla 30000, Algeria
authors' email: [1]ychebouat@gmail.com, [4]mbelfar@gmail.com

Received: 01/2023 **Published: 01/2023**

Abstract:

This study reported on the plant *Ephedra alata* known for its use in traditional medicine in Algeria, The work aimed to determine the antioxidant activity, phenol, and flavonoid contents of their Ethyl acetate extract and butanol extract.

Antioxidant activity was determined by the DPPH method, The essay of total phenols and flavonoid content were determined using the Folin-Ciocalteu reagent, and the Aluminum chloride colorimetric method respectively.

Obtained results show that the highest flavonoids and phenol contents were noted in Ethyl acetate extract.

the Ethyl acetate extract (46.66 ± 3.064 mg GAE/g extract) contains more phenolic compound compared to the Butanol extract (19.62 ± 0.244 mg GAE/g extract).

Additionally, concerning total flavonoid content Ethyl acetate extract (23.3 ± 00.504 mg QE/g extract). Meanwhile, Butanol extract (10.39 ± 0.193 mg QE/g extract).

The antioxidant activities are expressed as IC50 values. the Ethyl acetate extract showed an antioxidant activity capacity of 0.228 mg/ml. the Butanol extract had the lowest antioxidant capacity 0.581 mg/ml.

In this study, In general; the high content of antioxidant capacity indicated that they may impart health and nutritional benefit when involved in the food industry as a natural antioxidant.

Keywords: *Ephedra alata*, total phenolic, and flavonoid contents, antioxidant activity, DPPH method, Ethyl acetate extract, Butanol extract.

Tob Regul Sci.™ 2023;9(1): 251-259
DOI: doi.org/10.18001/TRS.9.1.17

Introduction :

In recent years, researchers have been interested in natural compounds that have important therapeutic and economic qualities, in particular phenolic compounds, because this family has been widely exploited in the field of phytotherapy due to its multiple therapeutic properties. the most important activity of these compounds is antioxidant activity [1].

Ephedra alata Decne. (the Arabic name is Alanda, family Ephedraceae) is a perennial genus of non-flowering seed herb belonging to the Gnetales plant, the closest living relative of the angiosperm.

The native land for this species in Iran, Algeria, Iraq, Chad, Egypt, Palestine, Lebanon, Jordan, Saudi Arabia, Morocco, Syrian Arab Republic, Libya, Mauritania, Mali, Somalia, and Tunisia.

Ephedra alata is a hardy, light green-branched, densely branched perennial shrub, 50-100 cm high and often wider than it is high. the twigs appear leafless and the leaves are reduced to small scales, cones sessile shaped, clustered in the axils or at branch tips .The Ephedra plant is strongly aromatic, with a bitter taste. The dried stem is the part of the shrub usually used for its therapeutic effects. E. alata grows wildly on the gravely rocky, sandy, and clay soil in arid environments often near shifting dunes. It has been used in traditional Chinese medicine for 5000 years to treat allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches, and nasal congestion.[2][3][4]

The imbalance between pro-oxidant and antioxidants is called oxidative stress. This is caused by a lack of antioxidants or by the accumulation of free radicals, primarily the reactive oxygen species (ROS) and the reactive nitrogen species (RNS), after the stimulation of the endogenous and external environment, oxidative stress could lead to cell death and the dysfunction of physiology, which could ascribe to DNA damage, and inflammation.

Synthetic antioxidants have a restriction for use, as they are suspected to be carcinogenic. [5]

It is in this sense that the study of the antioxidant activity of plants has now become important because we can find in plants powerful antioxidants.

The purpose of the present study was to investigate the antioxidant activity, phenol, and flavonoid content of *Ephedra alata*.

1.- Materials and Methods

Collection of Plant Material and extraction :

I had collected the plant Ephedra alata material from the flowering period in the Ouargla region (southeast Algeria).

before extraction, they were cleaned to eliminate soil and damaged seeds, dried for 15 days, and ground well into powder using grinder. from then soaked in a solution of ethanol - Eau (70%-30%) for 48 hours. the samples were then filtered through filter paper.

The filtrate was subjected to evaporation to leave the sample free of the ethanol using a rotary evaporator .

Extractions with solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, and, butanol. then filtered using filter paper (Whatman No. 1). The filtrates were concentrated using rotary evaporator.

- Determination of total phenolic content:

The assay of total phenolic content was carried out by the colorimetric method using Folin-Ciocalteu reagent and gallic acid as standard.

As a first step, Put 0.2ml of each extract into test tubes; Add 1 ml of Folin-Ciocalteu's reagent diluted 10 times in distilled water; Then leave to act for 5 minutes before adding 0.8 mL of 7.5% sodium carbonate. After 30 min of incubation at room temperature and protection from light, read the absorbance of a UV-vis spectrophotometer at 760 nm.

The same process is performed for gallic acid with different concentrations. The blank is represented by the solvent used added with Folin-Ciocalteu and Sodium Carbonate.

The same procedure was repeated for all standard Gallic acid solutions at different concentrations.

The TPC was calculated by a standard gallic acid graph, and the results were expressed as milligram gallic acid equivalent per 100g dry weight (mg GAE/100g DW).

Determination of total flavonoid content:

The total flavonoid content was determined by the colorimetric method using aluminum chloride reagent and Quercetin as standard.

in a test tube, we blended 1 ml of the sample solution with 1 ml of aluminum trichloride methanolic solution (2%). After stirring the test tubes, we let them rest for 10 to 15 min at room temperature. The same procedure was repeated for the standard Quercetin solutions at different

concentrations.

the absorbance of the reaction mixture was measured at 430 nm The TFC was calculated by a standard Quercetin graph, and the results were expressed as milligram quercetin equivalent per 100g dry weight (mg QE/100g DW).

Scavenging effect of the DPPH radical:

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical.

DPPH (diphenyl picryl hydrayl) is a relatively stable radical In these tests antioxidants reduce diphenyl picryl hydrayl having a violet color to a yellow compound, diphenyl picryl hydrazine, whose color intensity is inversely proportional to the capacity of the antioxidants present in the middle gave protons.

The DPPH solution is prepared by dissolving 2.44 mg of DPPH in 100 ml of methanol. 0.1 ml l of extract or standard solutions (quercetin) are added to 2.66 ml of DPPH, the mixture is left in the dark for 30 min, and the discoloration relative to the negative control containing only the DPPH solution is measured at 517 nm.

The antiradical activity is estimated according to the following equation:

% antiradical activity= [(Abs517 control-Abs517 sample)/Abs517control] x100

Determination of IC50 values:

The ability of anti-free radicals is determined by arithmetic terms in terms of the concentration of the solution to eliminate 50% of the free radicals. The result is expressed by:

IC50, which is defined by the concentration of the solution expressed in units (l / g) for crude extracts or in mm for pure compounds with known molar mass to clear %. 50 of DPPH roots, calculated from the curves of change in the percentage of inhibition in terms of the concentration of the solution, and the smaller the value of IC50, the greater the effectiveness of anti-radicals.

2.-Results and discussion:

- Phytochemical tests:

The phytochemical screening allowed us to highlight the presence of secondary metabolites in the plant tissues of our plant. The results obtained are collated in Table 1.

Table 1: Families of chemical compounds in the plant *Ephedra alata*.

Active ingredient	percentage of presence
Glycoside Flavonoids	+++
Flavonoids	+++
Free Flavonoids	+++
Alkaloids	+++
Cardenolides	++
Tannins	+++
Saponoids	+++
Steroids	++
Unsaturated sterols and terpenes	++
Unsaturated steroids	++

(+): Low presence (++): Medium presence
(+++): Strong presence (-): Absence

Considering the results obtained in the table: The plant is rich in active substances: flavonoids, Glycoside Flavonoids, Free Flavonoids, Alkaloids, tannins, and saponins. As for Cardenolides, Steroids, Unsaturated sterols, and terpenes, Unsaturated steroids present. in medium quantity.

Total phenolic content :

TPC contents are the process to figure out the amount of phenolic content in the samples. The phenolic level was determined by the Folin-Ciocalteu assay.

A calibration curve of standard reference was established using Gallic acid as a standard reference plotted. (standard curve equation Y = 3.429 x + 0.012 R 2= 0.990) .

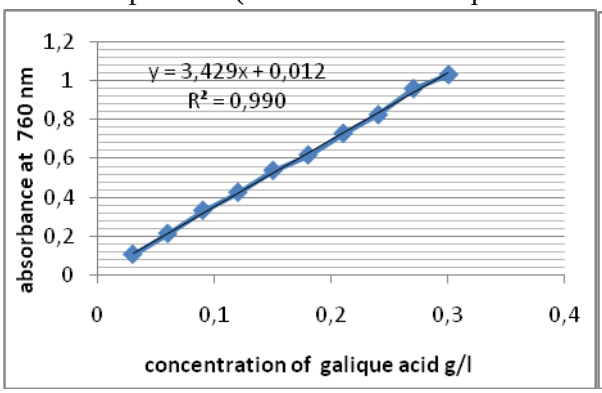


Figure 1 : Calibration curve of standard gallic acid for determination of total phenolics

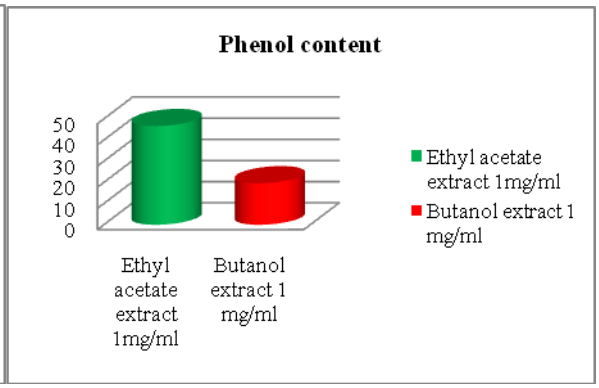
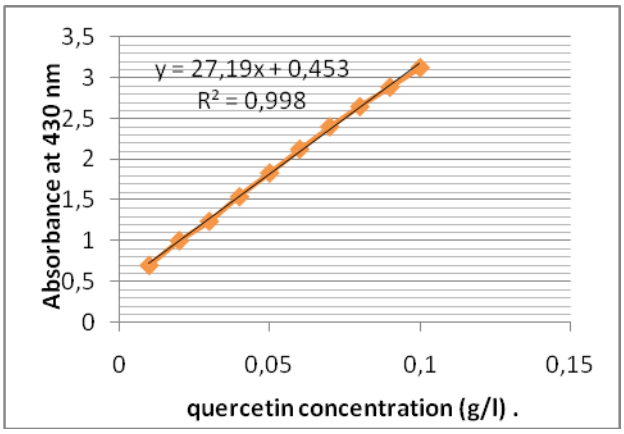


Figure 2 : Total phenolic of ethyl acetate extract and butanol extract

The highest phenol content was noted in the Ethyl acetate extract (46.66 ± 3.064 mg GAE/g extract). the Butanol extract (19.62 ± 0.244 mg GAE/g extract) .

Total flavonoids content :

Total flavonoids were determined using the modified aluminum chloride, where Quercetin was used as a reference standard to estimate flavonoid content. The standard curve equation (Y= 27.19 +0.453 R2 = 0.998).



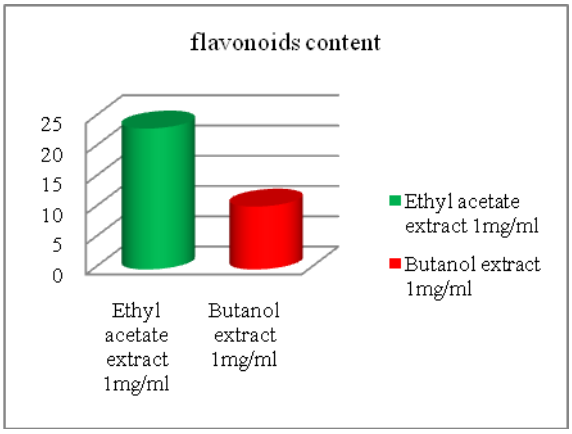


Figure 3:Calibration curve of standard Quercetin for determination of total flavonoid content.

Figure 4: Total flavonoid of Ethyl acetate extract and Butanol extract

According to the study, Ethyl acetate extract of Ephedra alata. showed the highest amount of total flavonoid content (23.3 ± 00.504 mg QE/g extract), while the Butanol extract had the lowest amount (10.39 ± 0.193 mg QE/g extract) total flavonoid content.

Table 2: phenol and flavonoid contents of Ethyl acetate extract and Butanol extract.

Extract	phenol content (mg GAE/g extract)	flavonoids content(mg QE/g extract)
Ethyl acetate extract	46.66 ± 3.064	23.3 ± 0.504
butanol extract	19.62 ± 0.244	10.39 ± 0.193

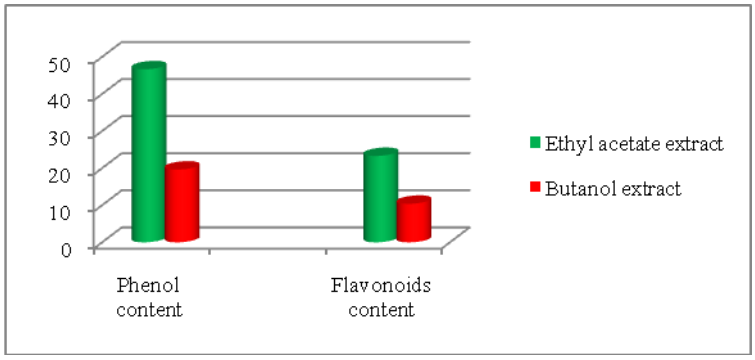
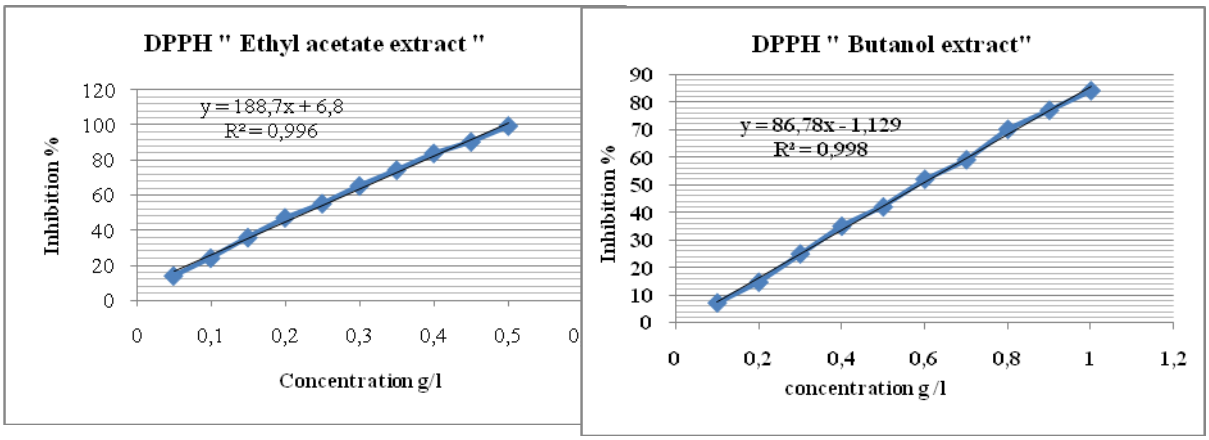


Figure 5: Total phenolic and total flavonoid contents of Ethyl acetate extract and Butanol extract.

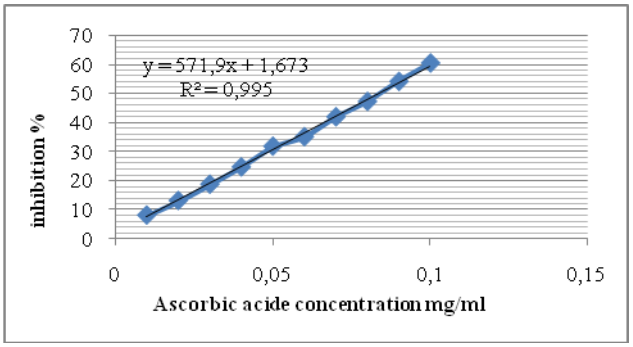
- Evaluation of antioxidant activity:

- Application of DPPH antiradical activity:

DPPH, a violet-colored free radical, is reduced to a yellow-colored compound in the presence of antiradical compounds. The intensity of the coloration, measured with a spectrophotometer, is inversely proportional to the antiradical activity of the different extracts whose activity is to be determined. Figure 6 shows the percentage inhibition of the DPPH radical as a function of the concentration of Ethyl acetate extract and Butanol extract.



(a) (b)



(c)

Figure 6: (a)- (b) – Scavenging effect of DPPH by Ethyl acetate extract and Butanol extract, respectively. **(c) -** Calibration curve for ascorbic acid (vitamin C**).**

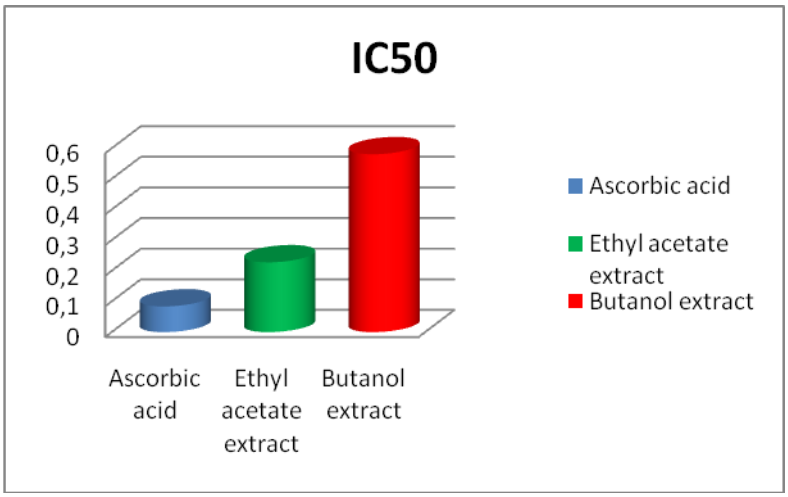


Figure 7: The IC50 values for ascorbic acid , Ethyl acetate extract and Butanol extract.

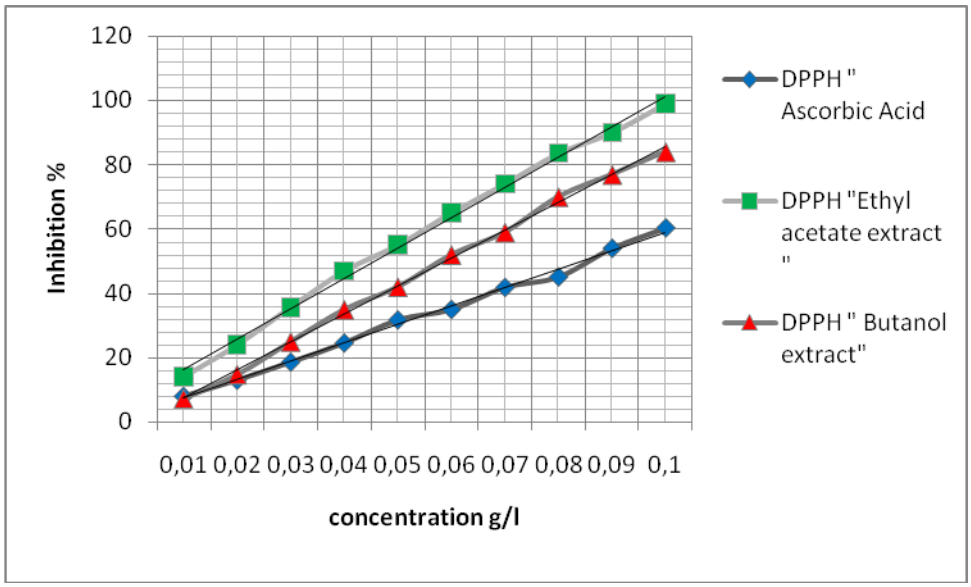


Figure 8: DPPH free radical scavenging activity of standard ascorbic acid , Ethyl acetate extract and Butanol extract.

We have determined concentration is Necessary to reduce 50% of the free radical DPPH or IC50 for ascorbic acid and each extract, The IC50 is inversely proportional to the antioxidant capacity of a compound because it expresses the number of antioxidants required to decrease the concentration of the free radical by 50%. The smaller the IC50 value, the greater the antioxidant activity for the fraction.

According to the results obtained, there is an increase in the percentage of inhibition proportional to the increase in concentration either for the standard (ascorbic acid) or for the different extracts of the plant. and we note that the IC50 of ascorbic acid is equal to 0.084 mg/ml, while the IC50 of extracts ethyl acetate and butanol are equal to 0.228 and 0.581 mg/ml respectively.

The determination of the antioxidant power of the extracts compared to that of ascorbic acid shows lower values of the extracts compared to that of ascorbic acid.

On the other hand, the result of the present study showed that the extract of acetate ethyl, which contains the highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity; the antioxidant ability center of phenolic acids is phenolic hydroxyl, so the number and position of phenolic hydroxyls are directly related to their antioxidant activity.

The phytochemical tests indicated the presence of flavonoids, Glycoside Flavonoids, alkaloids, and tannins in a plant. Several such compounds are known to possess potent antioxidant activity. Hence, the observed antioxidant activity may be due to the presence of any of these constituents.

Conclusion :

In the light of findings of the present study, particularly remarkable antioxidant activities and phenolic and flavonoids content properties of different extracts could trigger scientists' interest in the use of natural sources for food; Natural antioxidants that are present in plants are responsible for inhibiting or preventing the deleterious consequences of oxidative stress.

We also think that the results obtained in this study provide useful information for researchers who want to study various biological activities and encourage entrepreneurs to use them for commercial; use in pharmaceutical, cosmetics, and many other industrial fields, especially in the food industry with respect of biodiversity conversation.

References :

[1] Tungmunnithum D., Thongboonyou A., Pholboon A., and Yangsabai A. *Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview*. Medicines . 2018. 5(93) : 1-16.

[2] Jaradat N., Hussen F., and Anas Al Ali A. *Preliminary Phytochemical Screening, Quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of Ephedra alata Decne*. J. Mater. Environ. Sci. 2015. 6 (6) : 1771-1778.

[3] Gorai M., Laajili W., Santiago L.S., and Neffati M. *Rapid recovery of photosynthesis and water relations following soil drying and re-watering is related to the adaptation of desert shrub Ephedra alata subsp. alenda (Ephedraceae) to arid environments*. Environmental and Experimental Botany. 2015. 109 :113-121.

[4] AL-Snafi A. E. *Therapeutic importance of Ephedra alata and Ephedra foliate*. A review. IAJPS (Indo-American Journal of Pharmaceutical Sciences). 2017. 4 (2): 399-406.

[5] Dai J., and Mumper R.J. *Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties.* Molecules. 2010. 15 : 7313-7352.

[6] - Rohman A., Riyanto S., Yuniarti N., Saputra W. R., Utami R., and Mulatsih W. *Antioxidant Activity, Total Phenolic, and Total Flavaonoid of Extracts and Fractions of Red Fruit (Pandanus conoideus Lam)*. International Food Research Journal. 2010. 17 : 97-106.

[7] Amezouar F., Badri W., Hsaine M., Bourhim N., and Fougrach H. *Chemical Composition, Antioxidant and Antibacterial Activities of Leaves Essential Oil and Ethanolic Extract of Moroccan Warionia saharae Benth. & Coss.* Journal of Applied Pharmaceutical Science. 2012. 02 (05): 212-217.

[8] Saikia L. R., and Upadhyaya S. *Antioxidant Activity, Phenol and Flavonoid Content of some Less Known Medicinal Plants of Assam*. International Journal of Pharma and Bio Sciences. 2011 . 2(2): 383-388.

[9] Zuraini Z., Rais A., Yoga Latha L., Sasidharan S., and Xavier R . *Antioxidant Activity of Coleus Blumei, Orthosiphon Stamineus, Ocimum basilicum and Mentha arvensis from Lamiaceae Family*. International Journal of Natural and Engineering Sciences. 2008 . 2 (1): 93-95.

[10] Siddhuraju P. *The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (Vigna aconitifolia) (Jacq.) Marechal seed extracts.* Food Chemistry. 2006. 99(1): 149-157.

[11] Ardekani MRS., Khanavi M., Hajimahmoodi M., Jahangiri M., and Hadjiakhoondi A. *Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran*. Iranian journal of pharmaceutical research: IJPR, 2010. 9(2): 141.

[12] Pham T.T.H., Nguyen T.B.T., Nguyen T.N.T., and Vo Hoang K. *Total phenolic, total flavonoid contents and antioxidant potential of Common Bean (Phaseolus vulgaris L.) in Vietnam*. AIMS Agriculture and Food . 2020 . 5(4): 635–648.

[13] Akrout A., Gonzalez LA., El Jani H., and Madrid PC . *Antioxidant and antitumor activities of Artemisia campestris and Thymelaea hirsuta from southern Tunisia*. Food Chem. Toxicol. 2011. 49 : 342-347.