World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Evaluation of in-vitro Antioxidant and Anti-diabetic activities of leave aqueous extracts of Oudneya Africana

Zebidi Maroua, Seghiri Iman, Mehellou Zineb and Derouiche Samir*

Department of Cellular and Molecular Biology, Faculty of natural sciences sand life, University of El Oued, El-Oued 39000, Algeria

Received: 01-04-2018 / Revised Accepted: 23-04-2018 / Published: 01-05-2018

ABSTRACT

The aim of this study was to investigate the biological properties, including antioxidant, anti-diabetic activity and phytochemical analysis of Oudneya Africana R. Br.. Qualitative analysis of phytochemicals (flavonoid, alkaloid, saponins, steroids, phenol and carbohydrate) and quantitative analysis of total phenolics and flavonoids were prepared by using standard protocols. Anti-diabetic activity was estimated with Glucose uptake in yeast cells assay and Antioxidant activity was studied done DPPH assay. Results of Qualitative phytochemical analysis revealed that the aqueous extract show richness in flavonoids, saponins, phenols, steroids and carbohydrates and poor in alkaloids. Total phenol and flavonoid content show highest concentration in aqueous extract of O. Africana (19.35 mg GA EQ/gm, 6.43mg QEQ/gm). In vitro anti-diabetic and antioxidant studies show that aqueous extract of Oudneya Africana showed higher anti-diabetic property and important antioxidant activity with IC50 values was 45.41µg/ml in O. Africana. The results conclude that Oudneya Africana contains secondary metabolites compounds which have remarkable anti-diabetic and antioxidant activities. However additional comprehensive biological and pharmacological examination should be approved to isolate the active compounds to explain its mechanism of action to protect again antioxidant and diabetes mellitus.

Keywords: Oudneya Africana, Anti-diabetic, Antioxidant, Flavonoids, Phenols

Address for Correspondence: Derouiche Samir, Department of Cellular and Molecular Biology, Faculty of natural science sand life, University of El Oued, El-Oued 39000, Algeria;
Email: dersamebio@gmail.com

How to Cite this Article: Zebidi Maroua, Seghiri Iman, Mehellou Zineb and Derouiche Samir. Evaluation of Antioxidant and Antidiabetic activity of leave aqueous extracts of Oudneya Africana. World J Pharm Sci 2018; 6(5): 48-53.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Diabetes is associated with an extensive list of late complications involving nearly every tissue [1]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [2]. Medicinal plants are used throughout the world in the treatment of diabetes and cardiovascular pathologies. Some studies have shown that many plants are used in traditional medicine for their so-called hypoglycemic, lipid-lowering activities. and antioxidant [3].

Oxidative damage due to free radicals is associated with vascular disease in people with type 1 and those with type 2 diabetes mellitus (DM) [4]. Many herbal plants contains antioxidant compounds which protects cells against degenerative effects of Reactive Oxygen Species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxyl, radicals, hydroxyl radicals [5]. Oxidative stress is defined as an imbalance in the balance between antioxidants and pro-oxidants in favor of antioxidants Antioxidants play a major role in protecting against molecular oxidative damage [6]. Plants are important source for the discovery of new products of medicinal value for drug development and plants secondary metabolites are unique sources for pharmaceuticals food additives, [7]. So our objective in the present study is to evaluate the biological and pharmacological properties, including antioxidant, antidiabetic activity and phytochemical analysis of Oudneya Africana R. Br.

MATERIALS AND METHODS

Chemicals and reagents: All chemicals used were of analytical grade and purchased from Sigma-Aldrich, Mo, USA.

Collection and Extraction of Leaves Material: Fresh leaves of the plants were collected in October from a village in El Oued of El Oued state, Algeria. The leaves were washed with distilled water and used immediately. The extraction methods described by MAMTA and PARMINDER (2013) [8]. After extraction, the solvents were removed using rotary evaporator, to get gel-like extracts.

Phytochemical Screening: The methods of MAMTA and PARMINDER (2013) [8] were used to identify the phytochemicals provides in the extracts: alkaloids, saponins, tannins, steroids, flavonoids, terpenoids and glycosides.

Estimation of Total Phenol: The polyphenols are determined by the Folin-Ciocalteu method. This method, initially described by Slinkard and Singleton [9], makes it possible to know the total polyphenolic content of a given sample. The sample of the aqueous extract of the O. Africana (0.5 ml) and 2 ml of sodium carbonate (75 g / l) were added to 2.5 ml of 10% (v / v) Folin-Ciocalteau with gallic acid as standard. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm. The tests were carried out three times in order to ensure the reproducibility of the results. The total phenolic content was expressed in mg Equivalent of Gallic Acid per gram of sample.

Estimation of Total Flavonoids: Determination of the total flavonoid content of the aqueous extract of the O. Africana is carried out by the method described by Ahn et al. [10]. 0.5 ml of a 2% AlCl3-ethanol solution was added to 0.5 ml of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as a standard for plotting the calibration curve. The tests were carried out three times in order to ensure the reproducibility of the results. The results were expressed in milligram equivalent Quercetin per gram of sample.

Glucose uptake in yeast cells assay: Glucose uptake assay by using yeast cells was made according to the method of Cirillo et al., (1963)[11]. The commercial baker's yeast in distilled water was exposed to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were attained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of aqueous extract of O. Africana (50-250 µg/ml) were added to 1 ml of glucose solution (5 mmol) and incubated together for 10 min at 37 °C. The reaction was started by adding 100 µl of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and the total of glucose was estimated in the supernatant. Metformin was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula: Increase in glucose uptake (%)=Abs sample−Abs control÷Abs sample ×10 Where, Abs sample is the absorbance of the test sample, and Abs control is the absorbance of control reaction (containing all reagents except the test sample). All the experiments were carried out in triplicates.

In vitro Antioxidant activity Assay: The in vitro antioxidant activity was evaluated by measuring the scavenging power of the DPPH (1,1-diphenyl-2-picryhydrazyl) radical according to the method described by Burits and Bucar [12], where 3ml of various concentrations (5, 10, 15, 25,50, et 60µg/ml) of Oudneya Africana samples were added to 75µL of methanolic solution of DPPH (1.3mg/ml) . Absorbance measurements were read at 517 nm after 30 min of incubation time at room temperature (A1). Absorbance of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control (A0). The percentage inhibition [(A0-A1/A0) × 100] was plotted against the phenol content and IC50 was determined.

RESULTS AND DISCUSSION

Phytochemical Screening: The phytochemical screening results (Table 1) revealed the presence of a wide range of bioactive secondary metabolites including, phenol, saponins, flavonoids, tannins and carbohydrates and the absence of other bioactive substance such as alkaloids. Secondary metabolites produced by the oudneya africana possess a wide range of biological activities[13]. Phenolic compounds such as phenols, flavonoids and tannins are considered major contributors to the antioxidant capacity of plants [14]. These antioxidant compounds could have played a major role in scavenging the reactive oxygen species [15] which interest for the prevention and treatment of various diseases including cancers, inflammatory diseases, diabetes, osteoporosis, cardiovascular and neuro-degenerative diseases [16]. Plants containing chemical constituents having steroidal structure proved to be anti-inflammatory agents by modern clinical and pre -clinical studies. [17]. Glycosides and flavonoids can inhibit tumor growth and protection against gastrointestinal infections. Saponin is a substance characterized by its surfactant properties and cholesterol binding properties [18]. The presence of each secondary metabolite in Oudneya africana provides a rationale for the traditional use of these plants in the treatment of various health problems.

Table 01: Phytochemical composition of Aqueous extract of Oudneya africana
(+ presence, - absence)

Phytochemical	Leave Aqueous extract of O. Africana
Flavonoïdes	+++
steroids	++
Phénolique	+++
Tannin	+
Saponoside	+
Carbohydrate	++
Alcaloïde	-

Phenolic and Flavonoid Compounds: Phenolic compounds contain hydroxyl groups (-OH) that facilitate their free radical scavenging activity and act as antioxidants, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity [19]. In the other hand, Flavonoid shows antioxidant activity due to the presence of free -OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo [20].The Total Phenolic and Flavonoids Compounds was expressed in terms of gallic acid equivalents (mg of GAE/gm sample) and of Quercetin equivalents (mg of QE/gm sample) respectively, using the following equation based on the calibration curve: $Y = 0.0113x + 0.0686$ $R^2 = 0.998$ for phenolic compouneds and $Y = 0.035x + 0.288$ $R^2 = 0.995$ for flavonoids compouneds. Total phenolic and flavonoids contents of Oudneya africana obtained from water solvent is 19.53 mg GAE/gm and 6.43 mg of QE/gm respectively (Table 02). Phenolic compounds are well known as antioxidants and directed against free radicals associated with oxidative damage. Tannin and flavonoids act on the complications of diabetes by their antioxidant and anti-enzymatic properties, neutralizing the effect of free radicals and limiting the inflammatory reaction in different tissues [21]. Flavonoids are a group of natural compounds with variable phenolic structures and are found in plants [22]. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability [23].

Table 02: Total Phenol and flavonoids content

Compounds	**Total phenol content mg of GA eq/gm sample**	**Flavonoid content mg of Quercetin eq/gm dry wt**
Aqueous extract of Oudneya africana	19,35 ± 1,03	6,43 ± 0,08

Glucose uptake in yeast cells: In Glucose uptake in Yeast cells model the extracts of Oudneya africana leaves at different concentrations (50µg-250 µg) are subjected to in vitro glucose uptake assay using yeast as model. The percentage of glucose uptake in yeast cells by the extract was compared with Metformin standard drug. In Glucose uptake assay Oudneya africana extracts and standard showed dose dependent manner of activity i.e. as the concentration of sample increased even the percentage of inhibition also increases. Oudneya africana aqueous extract shown higher activity than the remaining extracts and then the Metformin standard drug ; results are shown in figure 1; The results indicate that aqueous extract of Oudneya africana shown appreciable anti-diabetic activity in performed in-vitro assays where as other tested extracts showed the least anti-diabetic activity. Plants are provided with secondary metabolites such as alkaloids, flavonoids, tannins, phenols and saponins which are also known as bioactive compounds and these phytochemical compounds possess different biological activities which include the anti-diabetic activity [24]. Flavonoid contents that were reported to have anti-diabetic activity, so it's substances accelerate the functioning of the intracellular enzymatic machinery, responsible for the uptake of extracellular glucose [25].

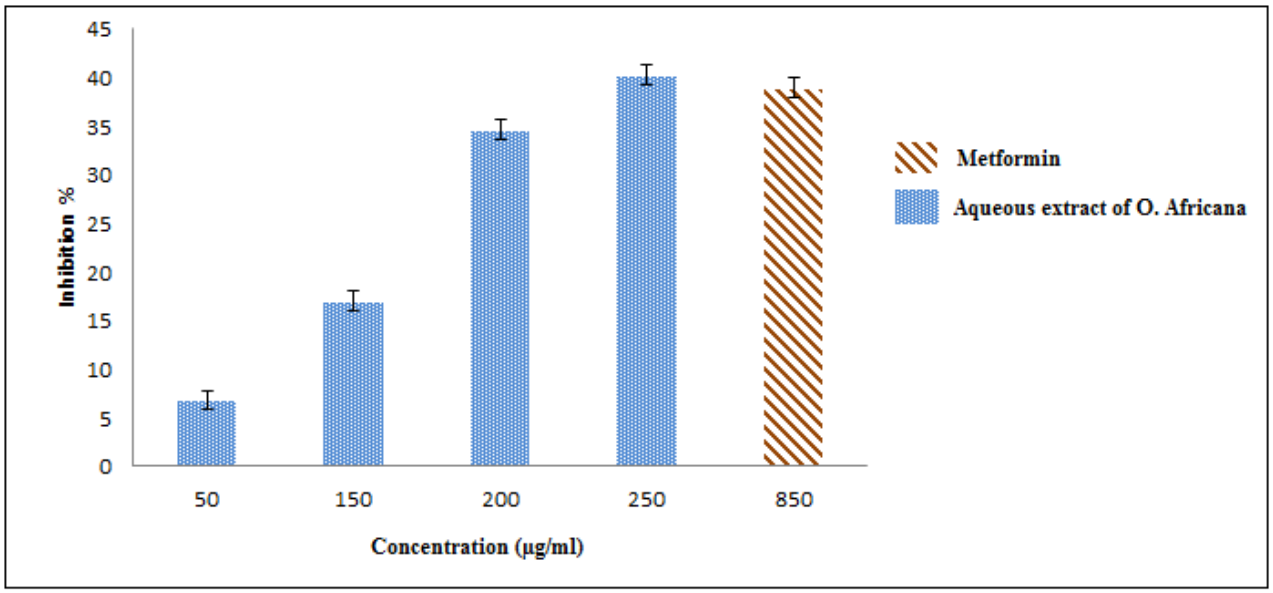

Figure 01: percentage of glucose uptake in yeast cells treated with Oudneya Africana extract

DPPH Antioxidant Activity and IC50 Value: The results of the experiment for antioxidant activity are shown in Fig. 02. The examination of antioxidant activity of extracts from O. Africana showed values varied from 15% To 95% of various concentrations. Reactive Oxygen species (ROS)/ Oxidants formed in our body due to exogenous and endogenous factors are found to be responsible for many diseases [26]. Now the research is going on to reveal the potential of phytochemical antioxidants as health benefactors. This is due to their ability to neutralize the free radicals or ROS or oxidants responsible for the onset of cell damage. Flavonoid and other phenolic compounds of plant origin have been reported in scavengers and inhibitors of lipid peroxidation [27, 28]. Figure 03 shows the IC50 values in the DPPH radical scavenging activity assay of the extracts. It was found that the antioxidant activity in O. Africana (IC50 = 45.41µg/ml). The IC50 of a compound is inversely related to its antioxidant capacity, as it expresses the quantity of antioxidant necessary to decrease the DPPH concentration by 50%, which is obtained by interpolation from a linear regression analysis [29]. A lower IC50 indicates a higher antioxidant activity of a compound and Huns [30].

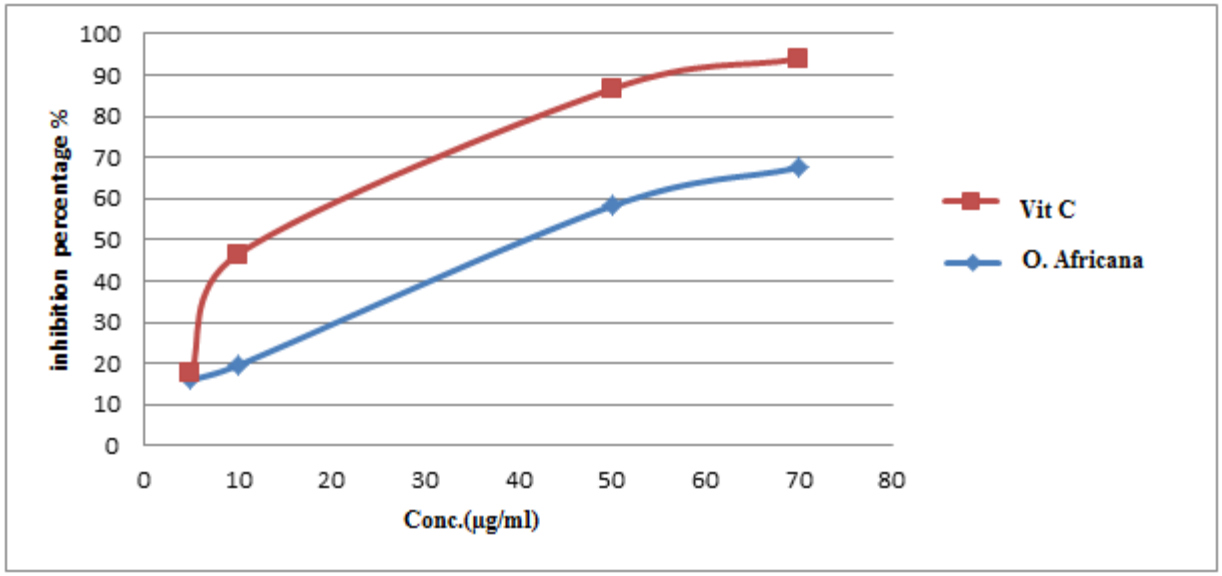

Figure 02: DPPH antioxidant activity of Oudneya Africana

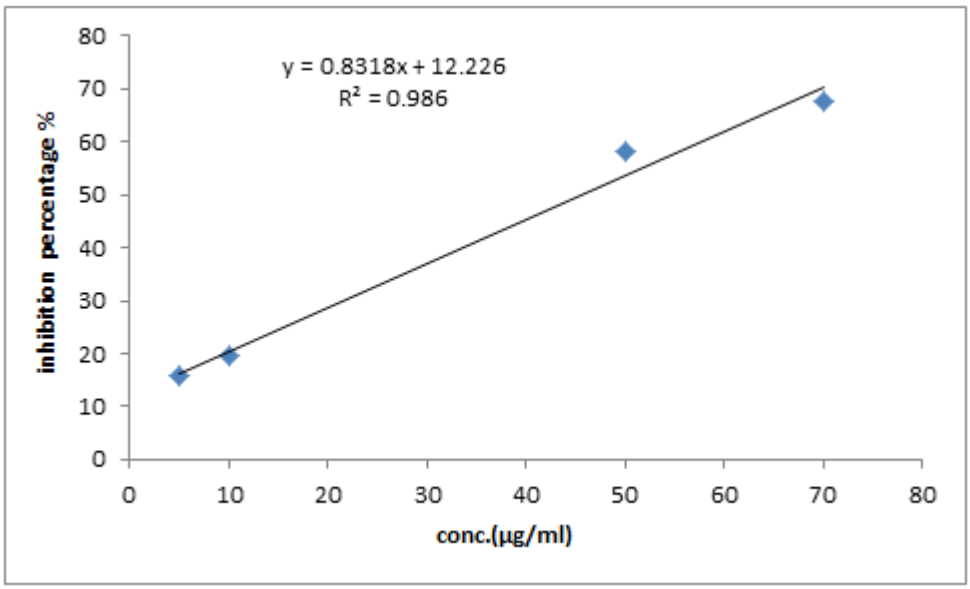

Figure 03 : IC50 VALUE of O. Africana

CONCLUSION

Phytochemical screening of leave aquous extracts of Oudneya Africana revealed the presence phenols, flavonoids, tannins, steroids, saponins and carbohydrates by positive reaction with the respective test reagent and absence of the alkaloids substance. Results obtained in this investigation indicate that the plant extracts of O. Africana rich in phenolics and exhibited highest antioxidant activities. The finding of this study suggest that this plant could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases.

ACKNOWLEDGEMENT

The first author would like to thank the Faculty of Sciences of Nature and Life, University of El Oued, Algeria for the permission to utilize the facilities to make this work. This work was supported by the research projectF03220140012 funded by the ministry of higher education, Algeria.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCE

1. Derouiche S et al. Beneficial Effect of Zinc on diabetes induced kidney damage and liver stress oxidative in rats. J Adv Biol 2017; 10(1): 2050-5055.
2. Campos C. Chronic hyperglycemia and glucose toxicity: pathology and clinical sequelae. Postgrad Med 2012;124(6): 90-97.
3. Fezan H et al. Studies of some therapeutic plants used in the treatment of arterial hypertension and diabetes: two emerging diseases in Cote d'Ivoire. Sci Nat 2008 ; 5: 39-48.
4. Derouiche S et al. Changes in metabolism of Zinc and carbohydrate and testis oxidative stress of diabetic rats fed zinc-over dose diet. Int J Biol Med Res 2017; 8(3): 6041-6045.
5. Lobo V et al. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010; 4(8): 118–126.
6. Derouiche S et al. Study of Oxidative Stress during Pregnancy. Glob J Pharmaceu Sci 2018; 4(5): 555646.
7. Ruby T, Rana CS. Plant secondary metabolites: a review. Int J Eng Res Gen sci ROAD 2015; 3(5): 661-670 .
8. Mamta A, Parminder K.. Phytochemical screening of orange peel and pulp. IJRET 2013; 2: 517-522.
9. Slinkard K, Singleton VL. Total Phenol Analysis: Automation and Comparison with Manual Methods. Am J Enology Vitic 1977; 28: 49-55.
10. Ahn MR et al. Antioxidant activity and constituents of propolis collected in various areas of China. Food Chem 2007; 101: 1383-1392.
11. Arun KS, Ankala BV. Evaluation of antidiabetic potential of kandelia candel and rhizophora apiculata- an in vitro approach. Int J Pharm Sci Res 2017; 8(6): 2551-2559.
12. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil, Phyt Res 2000; 14: 323-328.
13. Panchanathan M et al. Pharmaceutically active secondary metabolites of marine actinobacteria. Microbiol Res 2014;169 (4): 262-278 .
14. Mohan VR et al. Evaluation of antioxidant activity of aristolochia krysagathra (Aristolochiaceae)- an important medicinal herb. Inter J Pharm 2014; 4(1): 410-416.
15. Djouadi A, Derouiche S. Study of fluoride-induced haematological alterations and liver oxidative stress in rats. World J Pharm Pharm Sci 2017; 6(5): 211-221.
16. Keerthi M, Prasanna J, Aruna M, Rao N. Review on polyphenols as nature's gift world. J Pharm Pharmaceu Sci 2014; 3(4): 445-455.
17. Snehal S et al. Systematic review of plant steroids as potential anti -inflammatory agents: Current status and future perspectives. J Phytopharmacol 2015; 4(2):121-125.
18. Khalil AS et al.. Characterization of methanolic extracts of agarwood leaves . J App Industrial Sci. 2013; 1(3): 78-88.
19. Baba AS, Malik AS. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii. J Taibah Univ Sci 2015; 9(4): 449-454.
20. Geetha S et al. Evaluation of antioxidant activity of leaf extract of sea buckthorn (Hippophae rhamnoides L.) on chromium (VI) induced oxidative stress in albino rats. J Ethnopharmacol 2003; 87: 247-251.
21. Sunil K. The importance of antioxidant and their role in pharmaceutical science - a review. Asian J Res in Chem Pharm Scie 2014; 1(1):27 - 44
22. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 1998; 439:175-182,
23. Shashank K, Abhay KP. Chemistry and Biological Activities of Flavonoids: An Overview . Sci World J 2013; ID 162750
24. Mukherjee PK et al. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006; 106: 1-28.
25. Derouiche S et al. Effect of extracts aqueous of phragmites australis on carbohydrate metabolism, some enzyme activities and pancreatic islet tissue in alloxane induced diabetic rats. Int J Pharm Pharm Sci 2017; 9(6): 54-58.
26. Teraos KK et al. J Med chem. 1988; 37: 793-798.
27. Qaiyum AA et al. Extraction and determination of antioxidant activity of Withania somnifera Dunal. European J Exp Biol 2013; 3(5): 502-507.
28. Derouiche S et al. Polysaccharides and ascorbic acid content and the effect of aqueous extract of portulaca oleracea in high-fat diet-induced obesity, dyslipidemia and liver damage in albino wistar rats. Algerian J arid environ 2017; 7(2): 16-26.
29. Mahato G, Banerjee N. Phytochemical analysis and dpph antioxidant activity of two traditionally used plants occurring at purulia district ofwest bengal, india. Mahato and Banerjee . IJPSR 2017; 8(12): 5315-5319.
30. Abadi A, Hassani A. Essential oil composition and antioxidant activity of Marrubium vulgare L. growing wild in Eastern Algeria. Int Letters Chem Phys Astr 2013; 9(1): 17-24.