increasing phenolic content are benzene < chloroform < ethanol < methanol. The recovery, yield and types of phenolics compounds in an extract are influenced by the polarity of extraction solvents [32].

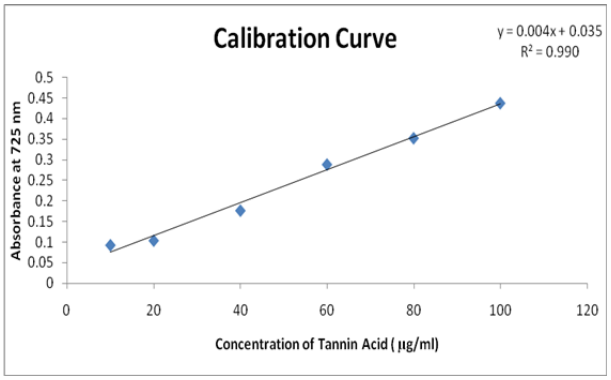

Fig.1: Tannic acid calibration curve

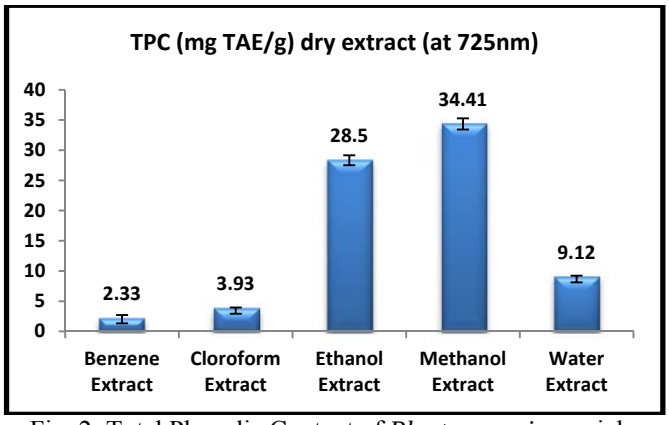

Fig. 2: Total Phenolic Content of *Plantago major* aerial part extracts

Each value shown in the Figure 2 was obtained by calculating the average of three experiments±SD.

3.2. ***In vitro* - antioxidant activity**

In the present study different solvent extracts of aerial part of *P. major* were investigated for antioxidant activity by using four different methods. The results of present showed that all the extracts exhibited antioxidant activity. Among all the extracts, methanolic extract showed significant antioxidant activity then the other extracts.

3.2.1. *DPPH free radical scavenging activity assay*

The DPPH free radical scavenging activity results of *P. major* aerial part extracts and BHT (standard antioxidant) are shown in figure 3.

The effect of antioxidant molecules on DPPH radical scavenging was due to their hydrogen donating ability. The antioxidant activity of plant extracts were calculated according to the percentage inhibition in DPPH assay. BHT was used as the standard antioxidant compound. The DPPH assay results of extracts in figure 3 shows that the methanol extract have better DPPH radical scavenging activity 80.67% at 400µg/ml as compared to ethanol (67.53%), chloroform (51.14%) and benzene (30.29%) extracts at same concentration. The DPPH free radical scavenging activity of methanol extract was compared with BHT (90.12%) at 400µg/ml. The qualitative and quantitative phytochemical screening results have confirmed the presence of polyphenolic compounds, which might be responsible for this activity. The free radical scavenging activity of all tested extracts is concentration dependent, if concentration increases DPPH radical scavenging activity also increases as shown in the figure 3. The IC50 values of polar and nonpolar extracts as given in the table 2 shows the lowest value of IC50 (139.19 µg/ml) for methanol extract which means lower the IC50 value, higher the antioxidant activity whereas ethanol (190.36 µg/ml), chloroform (341.80µg/ml) and benzene(691.93 µg/ml) extracts showed low antioxidant activity as their IC50 value were higher. The BHT has the lowest IC50 (99.95µg/ml). The comparison of IC50 values among extracts provides more understanding about the antioxidant potential of *P. major* extracts.

3.2.2. *Nitric Oxide Radical Scavenging Assay*

Nitric oxide radical scavenging activity results of aerial part extracts of *P. major* and BHT are shown in figure 4.

Nitric oxide radical scavenging activity of tested extracts was carried out at concentration of 10 to 400µg/ml. BHT was used as the standard antioxidant compound. The medicinal plants showed antioxidant activity through competing with oxygen to scavenge the nitrite radical. The percentage radical scavenging results shown in figure 4 reveal that nitric oxide radical scavenging activity increased with an increase in concentration of the tested extracts. Among all the tested extracts methanolic extract was the most potent nitric oxide radical scavenger. The scavenging activity of methanolic extract was 80.86% at 400µg/ml concentration as compared to ethanol (68.26%) chloroform (30.65%) and benzene (20.10%) extracts at same concentration. This activity of methanol extract was compared with BHT (98.69%) at 400µg/ml. The IC50 values of each examined extracts as shown in the table 2 shows the value in increasing order of IC50, methanol (124.16µg/ml), ethanol (201.38µg/ml), chloroform (728.26µg/ml) and benzene (1026.09µg/ml) extracts. The BHT has the lowest IC50 (76.15µg/ml). It is reported that lower the IC50 higher the antioxidant potential of the extracts [12].

3.2.3. *Hydrogen Peroxide Scavenging Activity Assay*

The antioxidant activity of plant extracts was calculated according to the percentage inhibition in H_2O_2 assay. The H_2O_2 scavenging activity results of aerial part extracts of *P. major* are shown in figure 5. The results obtained shows that methanolic extract have better H_2O_2 scavenging activity (83.27%) followed by ethanol (71.05%), chloroform (43.23%) and benzene (32.71%) extracts at 400µg/ml concentration. The scavenging activity of each extract was compared with BHT. The thorough study of chemical nature of the bioactive compound(s) is responsible for antioxidant activity which is still unknown. But preliminary phytochemical screening has confirmed the presence of polyphenolic compounds, which might be responsible for this activity. The H_2O_2 scavenging activity of extracts is concentration dependent, concentration increases activity also increases as shown in the figure 5. The IC50 value of each extract is given in the table 2 shows

the value in increasing order of IC50, methanol (148.20µg/ml), ethanol (198.16µg/ml), chloroform (450.10µg/ml) and benzene (622.66µg/ml) extracts. The BHT has the lowest IC50 (108.90µg/ml). It is reported that lower the IC50 higher the antioxidant potential of the extracts.

3.2.4 *Reducing Power Assay*

In this method, reducing ability of the tested extracts was measured by change of Fe^{3+} to Fe^{2+} form. Among all the extracts methanolic extract had high absorbance values than ethanol, chloroform and benzene extracts as shown in figure 6, this indicates that greater reducing and electron donor ability of methanol extract for breaking chain reaction and giving stable free radicals. Reducing power of all tested extracts and BHT (standard antioxidant) with respect to their absorbance values are shown in figure 6. The maximum absorbance of methanolic extract at 800µg/ml is comparable with BHT at 500µg/ml.

The *in-vitro* antioxidant results revealed that methanolic extract possessed maximum antioxidant potential while benzene extract showed least antioxidant activity in all the assays. When antioxidant activity of methanolic extract was compared with standard BHT, it was found that methanolic extract has lower antioxidant activity than the BHT.

The phytochemical compounds are capable of protecting the human being from the harmful effect of free radicals damage, which causes the oxidative stress. In the present study, the antioxidant potency of examined extracts were evaluated by four different methods, namely DPPH radical scavenging assay, NO scavenging assay, H_2O_2 scavenging assay and FRAP assay. IC50 of DPPH, NO and H_2O_2 scavenging activities of each examined extracts was calculated and compared to IC50 of BHT as shown in table 2.

Table 2: Antioxidant activity of aerial part extracts of *Plantago major*

Extracts	DPPH (IC50) µg/ml	NO (IC50) µg/ml	H_2O_2 (IC50) µg/ml
Benzene	691.93	1026.09	622.66
Chloroform	341.80	728.26	450.10
Ethanol	190.36	201.38	198.16
Methanol	139.19	124.16	148.20
Gallic acid	99.9	76.15	108.90

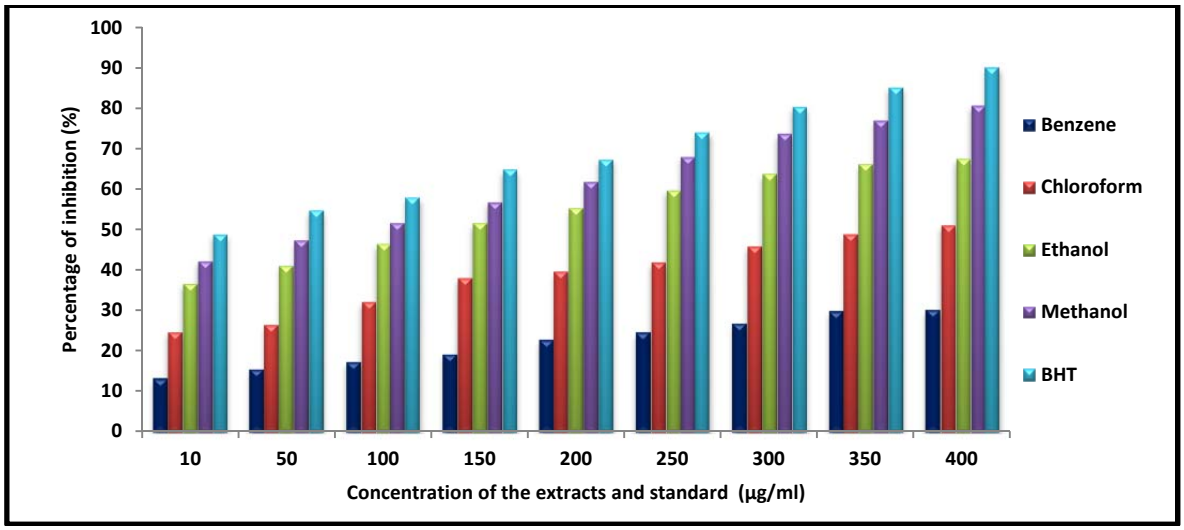

Figure 3: DPPH free radical scavenging activity of aerial part extracts of *p. major* and BHT

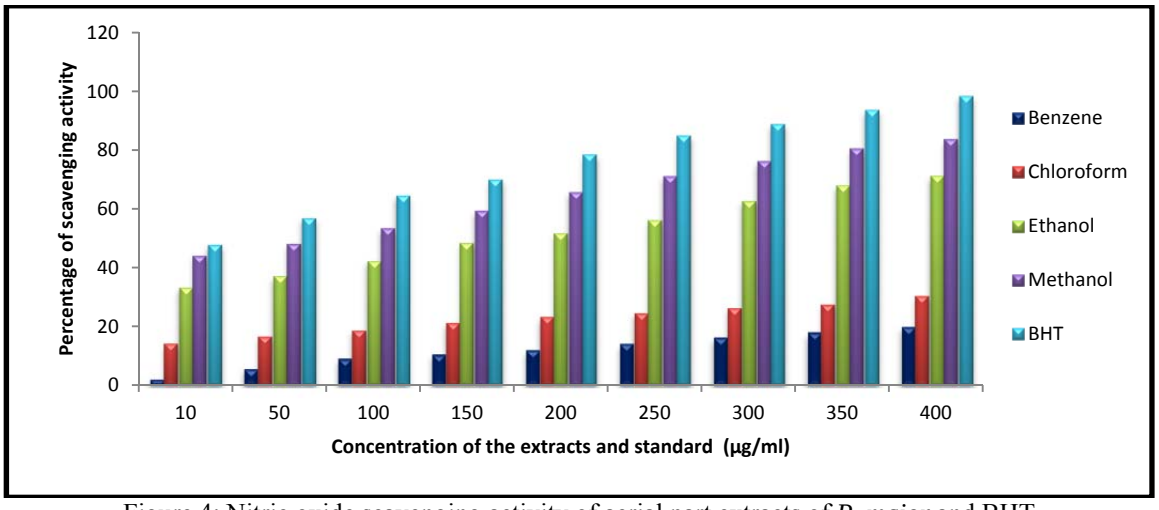

Figure 4: Nitric oxide scavenging activity of aerial part extracts of *P. major* and BHT

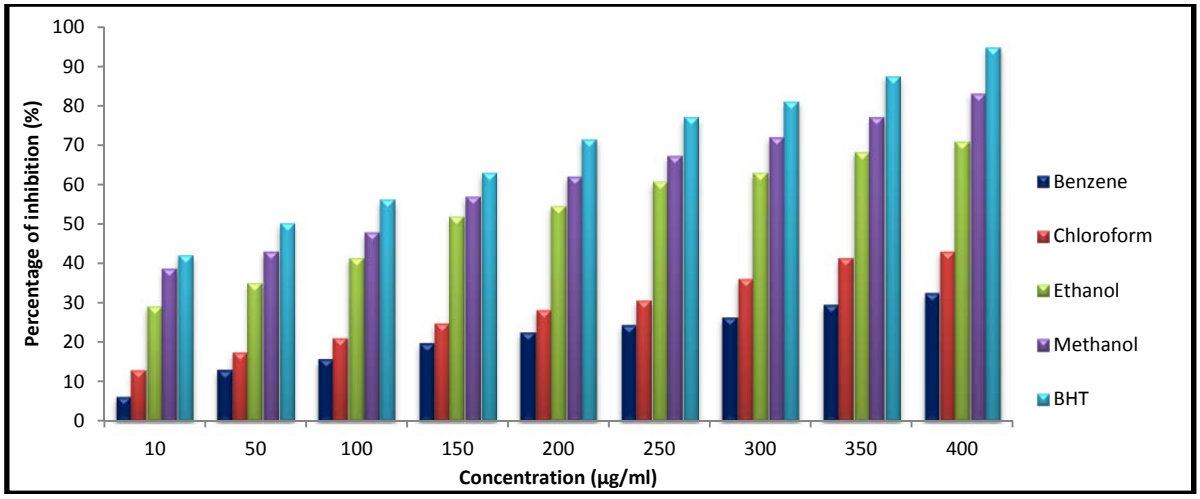

Figure 5: The hydrogen peroxide scavenging activity of aerial part extracts of *P. major* and BHT

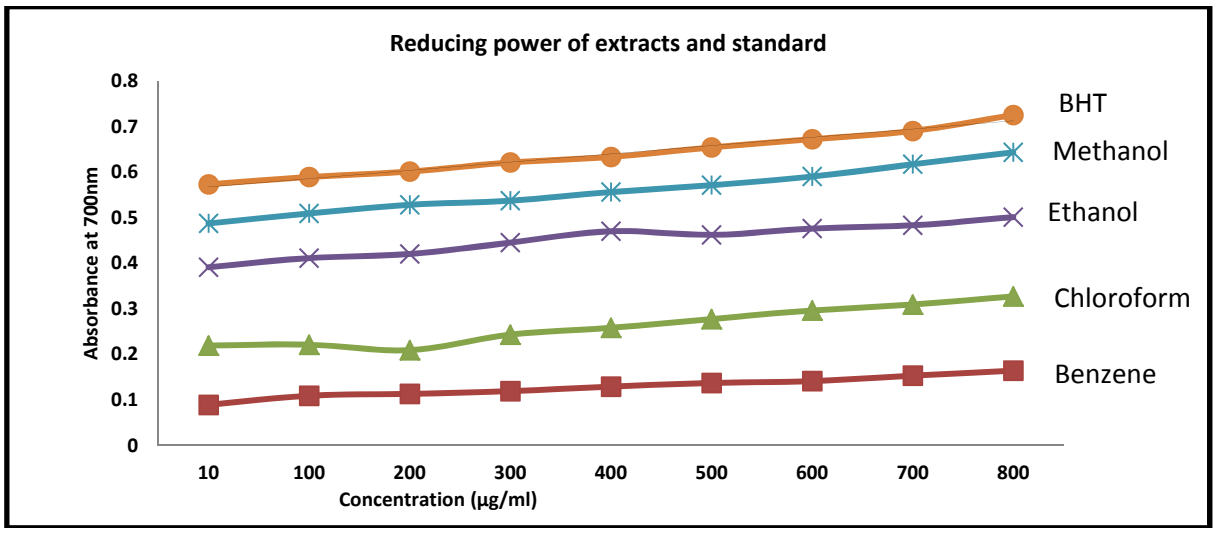

Figure 6: Reducing power of aerial part extracts of *P. major and BHT*

4. DISCUSSION

The TPC gives an idea about the phenolic compounds present in this plant which may be responsible for the pharmacological activities and the reason for their use as a traditional medicine in India and all over the world. The polarity of extraction solvent may influence the number of bioactive compounds obtained from the plant extracts. The results of TPC obtained in this study shows that polar solvents extracted more phenolic compounds than the non polar solvents. But in polar solvents, organic polar solvents (methanol and ethanol) extracted more phenolic compounds. It has been reported that methanol is found to be more desirable to extract the phytoconstituents. Metivier *et al*. reported that methanol was more effective as an extraction solvent for extracting the bioactive compounds from plant extracts and was 20% and 73% more effective than ethanol and water respectively [33]. Generally, the phenolic content of all the extracts were considerably high, which could be a major contributing factor to the strong antioxidant activity of plant extracts. According to Afolayan *et al*., [34], high phenolic content of plant extracts could be responsible for their antioxidant activity. In the present study, the antioxidant activity results were obtained showed that DPPH free radical scavenging activity of methanolic extract was highest than the ethanol, chloroform and benzene extracts. The nitric oxide and H_2O_2 scavenging activity results showed that scavenging activity by NO and H_2O_2 was correlated with the scavenging activity by DPPH assay. The results of present study are related with previous findings of antioxidant activity of *P major*. In ferric reducing power assay the reducing ability of each extract was depending on the electron donating power and ability to quench free radicals or terminate the free radical chain reaction by the formation of more stable products. The reducing agents delay the lipid peroxidation process because they donate a hydrogen atom for breaking the chain reaction which causes membrane lipid damage [35]. The capacity of phenolic compounds to scavenge free radicals may be due to phenolic hydroxyl groups they posses because they transfer hydrogen to free radicals and produce phenoxide radical, which is stabilized. The results of the study showed that among all the solvents, methanol was most efficient solvent for the extraction of polyphenolic compounds. This may be due to better

salvation of antioxidant compounds in aerial part extracts because of the interaction between the polar sites of the antioxidant molecules and methanol solvent.

The results of present study are in agreement with those obtained by Galvez *et al*., [36], Souri *et al.,* [37]; Beara *et al*., [16]. They reported that the phenolic compounds have been the main bioactive compounds present in the methanolic extract of selected Plantago species including *P. major* and are responsible for antioxidant activity of Plantago species. In this perspective Samuelsen, [5] reported that, *P. major* contains bioactive compounds such as flavonoids and phenolic compounds. Kobeasy *et al*., [38] also reported that, among three extracts namely ethanol, cold and hot water extracts, ethanolic extract of *P. major* leaves, had the highest total phenolic content, flavonoid and tannin content which is related to the highest antioxidant activity of ethanol extract of *P. major* leaves. Pourmorad *et al.*, [12] reported that methanolic extract of whole parts of *P. major* showed good radical scavenging activity which was comparable to BHT due to the presence of high amount of phenolic compounds was 31±4 mg of GAE/g of dry weight. The range of values of TPC was higher as reported in literature for *P. major* and also other species of Plantago such as *P. coronopus* and *P. lanceloata* [16].The differences noticed in the values of TPC could be related to different extraction methods or harvesting time and environmental features that can influence the concentration of phenolics in plantains [39].

Several studies reported that polyphenolic compounds may contribute antioxidant properties but some studies also reported that other than polyphenolic compounds may also contribute antioxidant activities. The study conducted by Reina *et al.* [6] concluded that ethanol extract of *Plantago major* and its two bioactive compounds baicalein and aucubin significantly reduced the production of reactive oxygen species by human neutrophils. It is reported in some studies that the natural products possessing antioxidant property shows liver protective activity [40]. Some earlier studies also reported that polar solvent extracts exhibited stronger antioxidant activity than nonpolar solvent extracts. This can be explained that bioactive compounds known for their antioxidant activity such as polyphenolic compounds which are soluble in polar solvents due to their polar nature [41]. Some studies also shows that there is a linear correlation between total phenolic content and antioxidant activity of sample. The results of this study shows that aerial part extracts of *Plantago major* may serve as a potential source of natural antioxidants. Thus this Plantago species can be suggested as a natural source of antioxidants as food and in pharmaceutical companies. Therefore, it can be used as dietary supplement to treat chronic diseases.

CONCLUSIONS

From this study it can be concluded that all the tested extracts showed antioxidant activity but among all the extracts, methanolic extract found out to be rich source of phytochemicals and exhibits highest amount of phenolic compounds and significant antioxidant activity on all examined antioxidant assays (DPPH, NO, H_2O_2 and reducing power). Therefore it can be stated that *P. major* is a potential source of natural antioxidant compounds may be useful for the development of newer and more potent natural antioxidant and can be used to prevent diseases related to oxidative processes. These results might be helpful for researchers and pharma companies for the development of valuable medicines which will be useful for treatment of various oxidative stress related diseases.

ACKNOWLEDGEMENTS

The authors are thankful to Honourable Vice-Chancellor of Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, U.P., India for providing all necessary facilities and Head, Department of Chemistry, SHUATS, Allahabad for providing necessary laboratory facilities to accomplish the experimental work.

ABBREVIATIONS

TPC - Total Phenolic Content
BHT – Butylated Hydroxyl Toluene
BHA – Butylated Hydroxyl Anisole
ROS – Reactive Oxygen Species
TAE – Tannic Acid Equivalent
FC – Folin Ciocalteu
DPPH – 1,1-diphenyl-2 picrylhydrazyl
IC50 – 50% Inhibition Concentration
NO – Nitric Oxide

REFERENCES

[1] Madhu, K., *Asian Journal of Pharmaceutical and Clinical Research*. 2013, 6(2), 38 - 42.
[2] Sadeghi, Z., Valizadeh, J., Shermeh, O. A., Akaberi, M., *Avicenna Journal of Phytomedicine.* 2015, 5(1), 1 - 9.
[3] Bhattacharyya, A., Chattopadhyay, R., Mitra, S., Crowe, S, E., *Physiological Review.* 2014, 94(2), 329 - 354.
[4] Shivasharanappa, K., Londonkar, R., *World Journal of Pharmaceutical Research*. 2014, 3(4), 2106 - 2116.
[5] Samuelsen, A, B., *Journal of Ethnopharmacology.* 2000, 71(1-2), 1 - 21.
[6] Reina, E., Al-Shibani, N., Allam, E., Gregson, K, S., Kowolik, M., Windsor, L, J., *Journal of Traditional and Complementary Medicine*. 2013, 3(4), 268 - 272.
[7] Tarvainen, M., Suomela, J., Kallio, H., Yang, B., *Chromatographia.* 2010, 71(3), 279 - 284.
[8] Mohmood, A, A., Phipps, M, E., *International Journal of Tropical Medicine*. 2006, 1(1), 33 - 35.
[9] Amini, M., Kherad, M., Mehrabani, D., Azarpira, N., Panjehshahin, M, R., Tanideh, N., *Journal of Applied Animal Research*. 2010, 37, 53 - 56.
[10] Zubair, M., Nybom, H., Lindholm, C., Brandner, J, M., Rumpunen, K., *Natural Product Research*. 2015, 30, 622 - 624.
[11] Guillen, M,E,N., Emim, J, A, S., Souccar, C., Lapa, A, J., *International Journal of Pharmacognosy*. 1998, 35(2), 99 - 104.
[12] Pourmorad, F., Hosseinimehr, S. J., Shahabimajd, N., *African Journal of Biotechnology*. 2006, 5(11), 1142 - 1145.
[13] Gomez-Flores, R., Calderon, C, L., Scheibel, L, W., Tamez-Guerra, P., Rodriguez-Padilla, C., Tamez-Guerra, R, *et al., Phytotherapy Research.* 2000, 14(8), 617 - 622.
[14] Chiang, L, C., Chiang, W., Chang, M, Y., Ng, L, T., Lin, C, C., *Antiviral Research.* 2002, 55(1), 53 - 62.
[15] Chiang, L, C., Chiang, W., Chang, M, Y., Lin, C, C., *American Journal of Chinese Medicine.* 2003, 31(2), 225.
[16] Beara, I, N., Lesjak, M, M., Jovin, E, D., Balog, K, J., Anackov, G, T., Orcic, D, Z, *et al*., *Journal of Agricultural and Food Chemistry.* 2009, 57(19), 9268 - 9273.
[17] Trease, G, E., Evans, W, C., *Pharamacognosy*, W, B, Scandars, Company, Ltd, London 1989.

[18] Sofowora, A., *Medicinal plants and Traditional Medicine in Africa*, John, Wily, and Sons, New York 1993.
[19] Harborne, J, B., *Phytochemical methods*: *A guide to modern techniques of Plant Analysis,* 3rd ed. Chapman, and Hall, London 1998.
[20] Makkar, H, P, S., Blummel, M., Borowy, N, K., Becker, K., *Journal of Science and Food Agriculture.* 1993, 61, 161-165.
[21] Wojdylo, A., Oszmianski, J., Czemerys, R., *Food Chemistry*. 2007, 105, 940 - 949.
[22] Ogunlana, O, E., Ogunlana, O, O., *Research Journal of Agriculture and Biological Sciences.* 2008, 4(6), 666 - 671.
[23] Marcocci, L., Maguire, J, J., Droylefaix, M, T., Packer, L., *Biochemical and Biophysical Research Communications*. 1994, 201(2), 748 - 755.
[24] Sreejayan, Rao, M, N, A., *Journal of Pharmacy and Pharmacology*. 1997, 49, 105 - 107.
[25] Ruch, R, J., Cheng, S, J., Klaunig, J, E., *Carcinogenesis*. 1989, 10, 1003 - 1008.
[26] Jayaprakash, G, K., Singh, R, P., Sakariah, K, K., J*ournal of Agricultural and Food Chemistry*. 2001, 55, 1018 - 1022.
[27] Oyaizu, M., *Japanese Journal of Nutrition.* 1986, 44, 307 - 315.
[28] Rao, A., Ahmad, S, D., Sabir, S, M., Awan, S., Shah, A, H., Khan, F, M., et al., *Journal of Medicinal Plants Research.* 2012, 7(4), 155 - 164.
[29] Do, Q, D., Anqkawijaya, A, E., Tran-Nquyen, P, L., Huynh, L, H., Soetaredjo, F, E., Ismadji, S., *et al*., *Journal of Food and Drug Analysis.* 2014, 22(3), 296 - 302.
[30] Pereira, C, G., Custodio, L., Rodrigues, M, J., Neng, N, R., Nogueira, J, M, F., Carlier, J., *et al*., *Brazilian Journal of Biology.* 2017, 77(3), 632 - 641.
[31] Stankovic, M, S., *Kragujevac Journal of Science.* 2011, 33, 63 - 72.
[32] Luque, de, Castro, M, D., Valcarcel, M., Tena, M, T., Analytical Supercritical Fluid Extraction, Butterworth Publishers, Boston 1994.
[33] Metivier, R, P., Francis, F, J., Clydesdale, F, M., *Journal of Food Science*. 1980, 45 (4), 1099 - 1100.
[34] Afolayan, A, J., Jimoh, F, O., Sofidiya, M, O., Koduru, S., Lewu, F, B., *Pharmaceutical Biology*. 2007, 45, 486 - 493.
[35] Loganayaki, N., Siddhuraju, P., Sellamuthu, M., *Journal of Food Science and Technology.* 2013, 50(4): 687 – 695.
[36] Galvez, M., Martin-Cordero, C., Houghton, P, J., Ayuso, M, J., *Journal of Agricultural and Food Chemistry.* 2005, 53(6), 1927 - 1933.
[37] Souri, E., Amin, G., Farsam, H., Barazandeh, Tehrani, M., *DARU Journal of Pharmaceutical Sciences*. 2008, 16(2), 83 - 87.
[38] Kobeasy, M, I., Abdel-Fatah, O, M., El-Salam, S, M, A., Mohamed, Z, E, M., *International Journal of Biodiversity and Conservation*. 2011, 3(3), 83 - 91.
[39] Tamura, Y., Nishibe, S., *Journal of Agriculture and Food Chemistry.* 2002, 50(9), 2514 - 2518.
[40] Hussan, F., Basah, R, H, O., Yusof, M, R, M., Kamaruddin, N, A., Othman, F., *Asian Pacific Journal of Tropical Biomedicine.* 2015, 5(9), 728 – 732.
[41] Iloki-Assanga, S, B., Lewis-Lujan, L, M., Lara-Espinoza, C, L., Gil-Salido, A, A., Fernandez-Angulo, D., Rubio-Pino, J, L., *et al*., *BMC Research Notes.* 2015, 8(396), 1 - 14.