Flavonoid/Phenol content Ratios in *Aspiliaafricana* (Wild sunflower) Methanolic Extracts: Implications for Oxidative Stress Management.

Abstract

Oxidative stress results from the imbalance between the production and regulation of reactive oxygen species or free radicals and causes harmful effects such as cancer, Alzheimer's disease, cardiac diseases, kidney disorders, liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders, aging and so on. Plant phenolics and flavonoids play a significant role in scavenging free radicals in the body and act as antioxidants. Hence, it is very important to estimate the total phenolic and flavonoid contents and the flavonoid/phenolic ratios in plants for the management of oxidative stress. Total phenolic and flavonoid contents of the extracts of Aspilia africana were determined using spectrophotometric methods. The results obtained showed that the highest total phenolic content value was observed for the roots extracts (150.75 ± 0.11 mg GAE/g) followed by the leaves extracts (112.17 ± 1.62 mg GAE/g), and the lowest was for the stems extracts (98.34 ± 0.19 mg GAE/g). The high phenolic content of the root (150.75 ± 0.11 mg GAE/g) indicates that the root of A. africana may have a high antioxidant capacity. The leaf of A. africana had the highest total flavonoid content while the stem had the lowest total flavonoid content. This is an indication that the extract has antioxidative potential. The F/P ratio ranges from 1.17 to 2.51, with the leaf extract having the highest flavonoid/phenolic ratio of 2.51 and the root extract having the lowest flavonoid/phenolic ratio of 1.17. The high F/P ratio of the leaves extract shows that it contains more rich flavonoids than the stems and roots. Hence, the leaf extract can be used in the management of oxidative stress.

Keywords: Total phenolic, total flavonoid, *AspiliaAfricana*, oxidative stress, antioxidant.

1.0 Introduction

Medicinal plants are the richest sources of phytochemicals and have been used in various traditional herbal practices since ancient times to treat numerous diseases associated with oxidative stress [4]. Phytochemicals are bioactive compounds obtained from plants [7]. These compounds are also known as secondary metabolites because the plants that produce them might have little need for them. They are naturally found in all parts of plants, including the bark, leaves, stems, roots, flowers, fruits, seeds, and other tissues [10].

Among the known classes of phytochemicals are phenolics such as polyphenols and flavonoids, which are significantly present in fruits, vegetables, and various medicinal and aromatic plants[9,22], and are widely recognised for their antioxidant properties [15, 16]. Antioxidants are substances that prevent or reduce oxidative damage to cellular components by neutralising free radicals, including peroxides and hydroperoxides[26]. This process helps lower the risk of diseases associated with oxidative stress [8, 12].

Oxidative stress itself is a condition characterised by an imbalance between the production of reactive oxygen species (ROS) or free radicals and the body's ability to effectively neutralise or repair the resulting damage [18, 20]. It may cause several severe human diseases such as cancer, Alzheimer's disease, cardiac diseases, kidney disorders, liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders, and aging [11].

Polyphenols are considered among the most bioactive compounds with exceptional antioxidant properties. They primarily function as antioxidants by scavenging free radicals and chelating metals in both in vitro and in vivo systems [6]. According to Alotaibi et al. [3], a high intake of phenolic compounds is associated with a reduced risk of cardiovascular diseases and cancers. Plant phenolics are known to exhibit a variety of biological activities, including anti-carcinogenic, antioxidant, antibacterial, anti-atherosclerotic, anti-inflammatory, and antiviral effects. Flavonoids are a prominent subgroup of phenolic compounds that have recently attracted significant interest due to their diverse pharmacological and biological activities. They are particularly well-known for their potent antioxidant properties [15, 25].

Aspiliaafricana is a semi-woody, low-toxicity herb belonging to the Asteraceae family and found predominantly in tropical regions. It is traditionally used to treat wounds and other ailments due to its richness in various bioactive compounds, such as flavonoids and phenolics [13, 17, 21]. Ajeigbe et al. [2] reported that *A. africana* possesses antimicrobial, hemostatic, anti-fertility, anti-ulcerogenic, antioxidant, and anti-inflammatory properties.

The current study aims to quantitatively determine the flavonoid-to-phenolic ratio in methanolic extracts of the leaves, stems, and roots of *A. africana.*

2.0 MATERIALS AND METHODS

2.1. Collection of plant material

The leaves, stems, and roots of *A. africana* were collected from abandoned building site in Oyigbo West, Oyigbo Local Government Area, Rivers State, Nigeria as shown in figure 1. The

plant was identified and authenticated by Dr. A.O Wokoma, Head of the Department of Biology, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. Figure 1 depicts *A. africana* thriving in an abandoned building site. This setting is particularly relevant as it highlights the plant's resilience and adaptability to harsh and disturbed environments. The ability of *A. africana* to grow in such a location suggests that the plant can endure stressful conditions, potentially leading to an increased production of secondary metabolites like phenolics and flavonoids, which are critical for its defense mechanisms.

Figure 1: Aspiliaafricana growing in an abandoned building site.

2.2 Preparation of plant extracts

The plant material was shade dried for about 15 days. The dried sample was then coarsely powdered and stored in a sterile container. The successive extraction of the samples from non-polar to polar solvents was done by using methanol using the standard technique of maceration. The extracts thus obtained were evaporated to dryness at room temperature and stored in a sterile airtight container. The concentrated mass obtained, i.e. the crude methanolic extracts were weighed and kept in a refrigerator for a further experimental procedure.

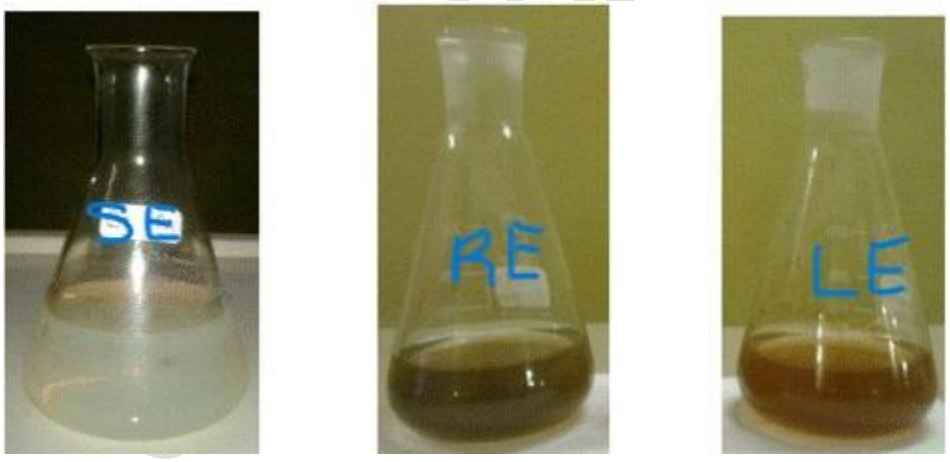

Figure 2. Pictures of different extracts of *A. africana*. SE=Stem extract, RE=Root extract, LE=Leaf extract

2.3.Spectrophotometric Estimation of Total Phenolic Content

The total phenolic content in the *A. africana* extracts was assessed using the Folin-Ciocalteu reagent, following the procedure outlined by Siddiqui et al. [24] with minor modifications. Gallic acid was employed as the reference standard to construct the calibration curve. A 0.5 ml aliquot of the plant extract (100 µg/ml) was combined with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and neutralized with 4 ml of a 7.5% sodium carbonate solution (w/v). The reaction mixture was incubated at room temperature for 30 minutes, with intermittent shaking to facilitate color development. The absorbance of the resultant blue color was measured at 765 nm using a UV-VIS spectrophotometer. The total phenolic content was calculated using the linear equation derived from a standard curve prepared with gallic acid and expressed as mg/g of gallic acid equivalent. The spectra for the estimation of total phenolic content are shown in Figure 3 below:

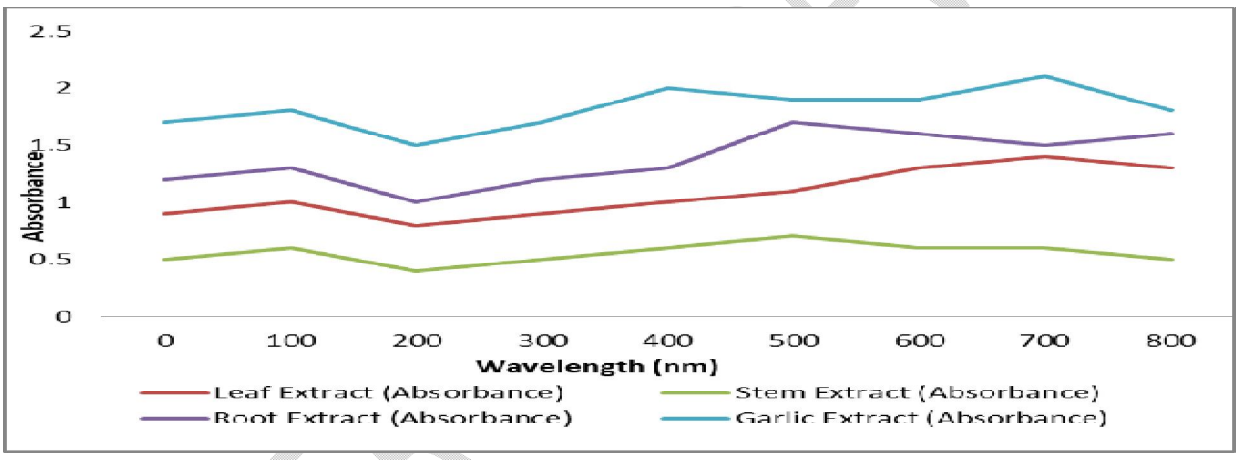

Figure 3. Uv-vis Spectra for the estimation of total phenolic content

2.4. Spectrophotometric Estimation of Total Flavonoid Content

The total flavonoid content in the *A. africana* extracts was determined using the aluminum chloride assay according to the method described by Ayele et al. (2022) with minor modifications. A 0.5 ml aliquot of the extracts was placed into separate test tubes, followed by the addition of 2 ml of distilled water. Next, 0.15 ml of sodium nitrite ($NaNO_2$, 5% w/v) was added and allowed to stand for 6 minutes. Then, 0.15 ml of aluminum chloride ($AlCl_3$, 10% w/v) was added and incubated for 6 minutes. Following this, 2 ml of sodium hydroxide (NaOH, 4% w/v) was added, and the volume was made up to 5 ml with distilled water. After a 15-minute

incubation period, the mixture turned pink, and its absorbance was measured at 510 nm using a spectrophotometer, with distilled water serving as the blank. The total flavonoid content was expressed as mg of quercetin equivalents per gram of extract. The spectra for the estimation of total flavonoid content are shown in figure 4 below:

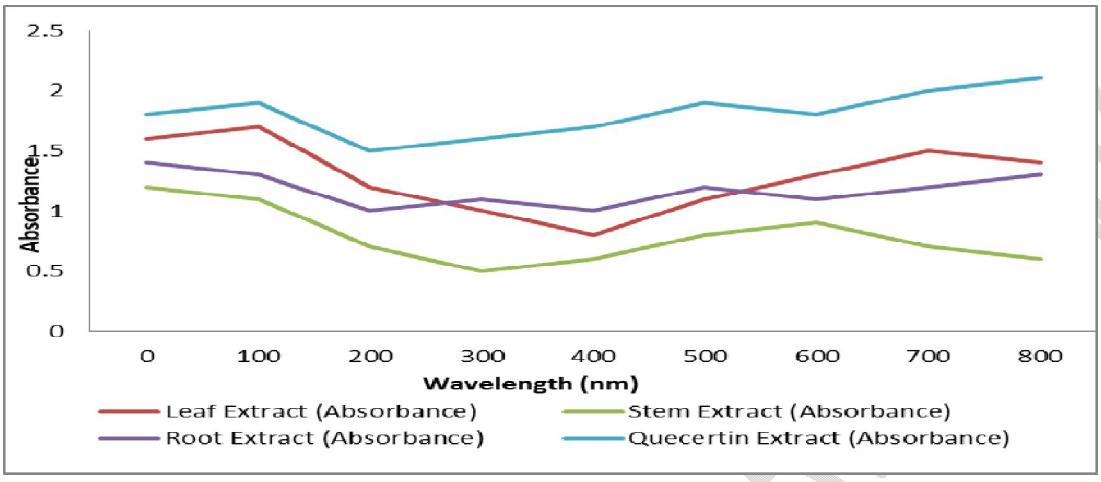

Figure 4. Uv-vis spectra for the estimation of total flavonoid content

3.0. Results and Discussion

Table 1. Total phenolic and flavonoid contents in leaf, stem, and root methanolic extracts of *Aspilia africana.*

Part of the plant	Total Phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Flavonoids/Phenolics (F/P ratio)
Leaves	112.17 ± 1.62	281.30 ± 0.20	2.51
Stems	98.34 ± 0.19	166.44 ± 1.43	1.69
Roots	150.75 ± 0.11	176.14 ± 0.35	1.17

3.1. Total phenolic content (TPC)

Total phenolic contents in different extracts of leaves, stems, and roots of *A. africana* were determined by Folin–Ciocalteu (F–C) method using gallic acid as the standard. The absorbance values gotten at different concentrations of gallic acid were used to determine the calibration curve. Total phenolic content of the extracts was calculated from the regression equation of the calibration curve ($Y = 0.0104x$; $R^2 = 0.988$) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) where y is the absorbance at 765 nm and x is total phenolic content in the extracts of *A. africana* extract expressed in mg/g equivalent of Gallic

acid. TPC values varied between 98.34 ± 0.19 and 150.75 ± 0.11 mg gallic acid equivalent per gram of extract as shown in Table 1. TPC values were higher in the roots and leaves extracts than in the stem extracts. The highest TPC value was observed for the roots extracts (150.75 ± 0.11 mg GAE/g) followed by the leaves extracts (112.17 ± 1.62 mg GAE/g), and the lowest was for the stems extracts (98.34 ± 0.19 mg GAE/g). The high phenolic content of the root (150.75 ± 0.11 mg GAE/g) indicates that the root of *A. africana* may have a high antioxidant capacity. This agrees with Phuyal et al. [19], who opined that the phenolic content of the part of any plant is directly related to its antioxidant properties. This implies that the roots of *A. africana* have high antioxidant properties than the other parts studied. Antioxidants are extremely important substances, which can defend the body from impairment caused by free radical-induced oxidative stress [25].

3.2. Total flavonoid content

The content of total flavonoid of the methanolic extracts of the three different parts of the plant measured spectrophotometrically by using the aluminum chloride assay is shown in Table 1. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the extract. The total flavonoid content varied between 166.44 ± 1.43 and 281.30 ± 0.20 mg quercetin equivalent per gram of extract. The leaf of *A. africana* had the highest total flavonoid content while the stem had the lowest total flavonoid content.

A study carried out by Ajeigbe et al. [2] has shown that leaf extract of *A. africana* significantly increased malondialdehyde (MDA) levels and decrease superoxide dismutase (SOD) and catalase (CAT) activities in experimental rats. This is an indication that the extract has antioxidative potential which is attributed to the presence of flavonoids in the extract, thus supporting my present findings.

3.3. Flavonoid/Phenol (F/P) Ratio

The calculated flavonoid/phenolic ratio to ascertain the extracts that are rich in flavonoids is shown in Table 1. The F/P ratio ranges from 1.17 to 2.51, with the leaf extract having the highest flavonoid/phenolic ratio of 2.51 and the root extract having the lowest flavonoid/phenolic ratio of 1.17.

This high F/P ratio of the leaf extract shows that it contains more rich flavonoids than the stem and roots. This agrees with the study carried out by Agbo et al. [1], who investigated the

antioxidant, total phenolic contents, and total flavonoid contents of selected Nigerian medicinal plants and observed that the leaves extracts of the plants studied had higher F/P ratio than the stem, although the roots of the selected plants were not included in the study. To the best of my knowledge, no flavonoid/phenolic ratio in *A. africana* extracts has been reported.

4.0. Conclusion

Methanolic extracts of the leaves, stems, and roots of *A.africana* were investigated for their total flavonoid contents, total phenolic contents, and flavonoid/phenolic content ratio. The leaf extract was found to be rich in flavonoids since it had high flavonoids/phenolics ratio. Hence, the leaf extract can be used in the management of oxidative stress.

5.0. Implication of the study on the management of oxidative stress

Oxidative stress is associated with numerous health issues, including cardiovascular diseases, cancer, neurodegenerative disorders, and diabetes [12]. For instance, in cardiovascular diseases, oxidative stress can lead to the oxidation of low-density lipoprotein (LDL), contributing to the formation of atherosclerotic plaques. In cancer, oxidative stress can cause mutations in DNA, leading to uncontrolled cell growth and tumour formation. Similarly, in neurodegenerative disorders such as Alzheimer's disease, oxidative stress can result in neuronal damage and cognitive decline [20, 23].

Moreover, oxidative stress is a significant factor in aging, accelerating the aging process and contributing to age-related diseases. The continuous oxidative damage to cellular components over time can lead to a gradual decline in cellular function and integrity, manifesting as aging symptoms and age-associated diseases [14].

The findings of this study regarding the flavonoid/phenolic content ratios in methanolic extracts from the leaves, stems, and roots of *A. africana* (Wild Sunflower) have significant implications for managing oxidative stress. The high ratio observed in the leaf extracts indicates a potent antioxidant capacity, suggesting that these extracts may offer considerable benefits in reducing oxidative stress. This potential makes *A. africana* leaves a valuable contender for developing antioxidant therapies aimed at mitigating the harmful effects associated with oxidative stress.

The study also highlights the potential of *A. africana* leaf extracts in managing diseases linked to oxidative stress, such as cardiovascular conditions, cancer, neurodegenerative disorders, and diabetes.

The leaf extracts of *A. africana*, with their elevated flavonoid content, could be used in developing dietary supplements or therapeutic formulations aimed at managing oxidative stress and enhancing overall health. This practical application of the study's findings underscores the importance of harnessing natural sources of antioxidants.

Above all, the study points to the need for further research to elucidate the specific mechanisms through which flavonoids and phenolics exert their antioxidant effects. Such research could help optimize the use of *A. africana* extracts in managing oxidative stress and improving health outcomes related to oxidative damage.

References

1. Agbo, M. O., Uzor, P. F., Nneji, U. N. A., Odurukwe, C. U. E., Ogbatue, U. B., &Mbaoji, E. C. (2015). Antioxidant, total phenolic and flavonoid content of selected Nigerian medicinal plants. *Dhaka University Journal of Pharmaceutical Sciences*, *14*(1), 35-41.
2. Ajeigbe, K. O., Onifade, A. A., Omotoso, D. R., Enitan, S. S., & Olaleye, S. B. (2014). Anti-ulcerogenic activity of Aspiliaafricana leaf extract: roles of gastric acid, oxidative stress and neutrophil infiltration. *African Journal of Biomedical Research*, *17*(3), 193-201.
3. Alotaibi, B. S., Ijaz, M., Buabeid, M., Kharaba, Z. J., Yaseen, H. S., & Murtaza, G. (2021). Therapeutic effects and safe uses of plant-derived polyphenolic compounds in cardiovascular diseases: a review. *Drug design, development and therapy*, 4713-4732.
4. Ashraf, M. V., Khan, S., Misri, S., Gaira, K. S., Rawat, S., Rawat, B., ... & Ahmad, S. (2024). High-Altitude Medicinal Plants as Promising Source of Phytochemical Antioxidants to Combat Lifestyle-Associated Oxidative Stress-Induced Disorders. *Pharmaceuticals*, *17*(8), 975.
5. Ayele, D. T., Akele, M. L., & Melese, A. T. (2022). Analysis of total phenolic contents, flavonoids, antioxidant and antibacterial activities of Croton macrostachyus root extracts. *BMC chemistry*, *16*(1), 30.

6. Bhuyan, U., &Handique, J. G. (2022). Plant polyphenols as potent antioxidants: Highlighting the mechanism of antioxidant activity and synthesis/development of some polyphenol conjugates. *Studies in Natural Products Chemistry*, *75*, 243-266.
7. Demir, T., & Akpınar, Ö. (2020). Biological activities of phytochemicals in plants.
8. Engwa, G. A., Nweke, F. N., &Nkeh-Chungag, B. N. (2022). Free radicals, oxidative stress-related diseases and antioxidant supplementation. *Alternative Therapies in Health & Medicine*, *28*(1).
9. Garg, S. K., Shukla, A., & Choudhury, S. (2019). Polyphenols and flavonoids. *Nutraceuticals in veterinary medicine*, 187-204.
10. Gebreslassie, H. B., & Eyasu, A. (2019). Phytochemical screening of the leaves Calpurnia aurea (Ait.) Benth extract. *Int. J. Clin. Chem. Lab. Med*, *5*, 18-24.
11. Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., Pramodh, S., Alsulimani, A., Alkhanani, M. F., Harakeh, S., Hussain, A., Haque, S., & Reshi, M. S. (2022). Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. *Cells*, *11*(3), 552. https://doi.org/10.3390/cells11030552
12. Kıran, T. R., Otlu, O., & Karabulut, A. B. (2023). Oxidative stress and antioxidants in health and disease. *Journal of Laboratory Medicine*, *47*(1), 1-11.
13. Komakech, R., Matsabisa, M. G., & Kang, Y. (2019). The wound healing potential of Aspiliaafricana (Pers.) CD Adams (Asteraceae). *Evidence□Based Complementary and Alternative Medicine*, *2019*(1), 7957860.
14. Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical interventions in aging*, *13*, 757–772. https://doi.org/10.2147/CIA.S158513
15. Makhaik, M. S., Shakya, A. K., & Kale, R. (2021). Dietary phytochemicals: As a natural source of antioxidants. *Antioxidants-benefits, sources, mechanisms of action*, 646.
16. Nwozo, O. S., Effiong, E. M., Aja, P. M., &Awuchi, C. G. (2023). Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. *International Journal of Food Properties*, *26*(1), 359-388.

17. Okello, D., Lee, J., & Kang, Y. (2020). Ethnopharmacological potential of Aspiliaafricana for the treatment of inflammatory diseases. *Evidence□Based Complementary and Alternative Medicine*, *2020*(1), 8091047.
18. Phull, A. R., Nasir, B., ul Haq, I., & Kim, S. J. (2018). Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. *Chemico-biological interactions*, *281*, 121-136.
19. Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of Zanthoxylum armatum DC. *The Scientific World Journal*, *2020*(1), 8780704.
20. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative medicine and cellular longevity*, *2017*, 8416763. https://doi.org/10.1155/2017/8416763
21. Popoola, O. K., Adekeye, K. D., Akinbinu, E. D., Adekeye, L. T., Afolayan, M. B., Bakare, E. A., & Akande, O. E. (2021). Ethnobotanical plants and their tradomedicinal values: A review. World Journal of Biology Pharmacy and Health Sciences, 5(1), 066-088.
22. Prabhu, S., Molath, A., Choksi, H., Kumar, S., & Mehra, R. (2021). Classifications of polyphenols and their potential application in human health and diseases. *Int. J. Physiol. Nutr. Phys. Educ*, *6*(1), 293-301.
23. Reddy, V. P. (2023). Oxidative stress in health and disease. *Biomedicines*, *11*(11), 2925.
24. Siddiqui, N., Rauf, A., Latif, A., & Mahmood, Z. (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). *Journal of Taibah university medical sciences*, *12*(4), 360-363.
25. Tariq, L., Bhat, B. A., Hamdani, S. S., & Mir, R. A. (2021). Phytochemistry, pharmacology and toxicity of medicinal plants. *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*, 217-240.
26. Yadav, A., Kumari, R., Yadav, A., Mishra, J. P., Srivatva, S., & Prabha, S. (2016). Antioxidants and its functions in human body-A Review. *Res. Environ. Life Sci*, *9*(11), 1328-1331.