Effects of Various Drying Methods on the Proximate Composition and Antioxidant Activities of *Stellaria media* Leaves

ABSTRACT

Background:*Stellaria media* (SM) belongs to the family of Caryophyllaceae. The plant is widely distributed in cold and temperate region all over the world. However, different parts of the SM plant have been used to treat various disorders such asdiarrhoea, jaundice, asthma, gastrointestinal disorders, measles, renal, reproductive, digestive, and respiratory tracts inflammations. Aims: In present investigation is aimed to determine the proximate and antioxidant activity of SM leaves by using three different drying methods- Sun drying (SD), Shadow drying (SHD), and Oven drying (OD).

Methodology: All the experiments were done according to standard procedures of the Association of Analytical Communities (AOAC).

Results: The result was compared with the fresh leaves (FL) of the SM. FLof SM was recorded to have maximum amount of moisture content. Also, the ash content which was determinant of minerals content was higher in OD sample compared with the FL and other drying methods. Protein and crude fiber were higher in dried samples as compared with the FL. Furthermore, levels of calcium (Ca) and iron (Fe) were higher in SD sample as compared with FL, SHD, and OD samples. Meanwhile, thetotal flavonoid content (TFC) was highest recorded in OD sample in methanol extract. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) showed a broad range of variation among the fresh or drying samples as well as solvent types. Also, the result showed in both DPPH and FRAP highest value in OD sample in methanol extract as same as TFC.

Conclusion: Nutrients and antioxidants help to maintain the nutritional status as well as to prevent various diseases. Therefore, they should be included as a dietary supplement.

Keywords*: Stellaria media, Proximate, Antioxidants, Nutrients, Medicinal plant, Protein*

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1. INTRODUCTION

Medicinal herbs are valued as the safest option for treating various chronic diseases since ancient times due to their higher therapeutic potential without any side effects compared to synthetic drugs [1]. The genetic potential of human beings can be improvised by using medicinal herbs as a good source of nutrition[2]. Around 3.3 billion people in developing countries depend on medicinal herbs for

basic needs[3]. *Stellaria media*(SM) is a perennial herb that belongs to the Caryophyllaceae family and contains 2630 species and 85 genera[4]. This herb growsin the moderate region and cool seasons of Asia, Europe, and North America. In India, it grows in the Himalayas up to the height of 4300 m. SM naturally grows in open fields that do not require extensive human efforts for cultivation[5]. SM germinates in the season of autumn and flower blooms in May and October. The SM leaves are opposite, simple, oval in shape with a smooth surface and green-yellow in color[6].

Traditionally, SM leaves decoction was used for medical applications. In Africa and Asia, leaves are used for the treatment of the gastrointestinal and respiratory tract infection. In India, leaves are used as a folk medicine for healing wounds, stop bleeding, pain killer, cancer, and inflammation. Leaves of SM possess several pharmacological activities such as anti-obesity, anti-bacterial, anti-viral, anti-fungal, and anti-hepatitis. The dried leaves are used for making pills, and powder to treat swelling in legs, infections, heart diseases, lung infections, and haemorrhoids[7]. Furthermore, SM leaves are rich in protein, fiber, carbohydrates, and minerals[8]. Nutrient-dense leaves of SM contain 16 essential amino acids of totally free and bound amino acids. SM leaves are also appraised to have more than 50 bioactive compounds that are responsible for their pharmacological properties[9]. The estimation of the bioactive compounds in SM leaves revealed beneficial phytochemical and antioxidant. Some of the significant phytochemical such as flavonoids, flavanol, alkaloids, terpenoids, saponins, phenol, phenolic compounds, phlobatannins, and glycosides are present in SM leaves[10]. Along with these, SM leaves are rich in antioxidant activity that are total phenolic content, diphenyl picrylhydrazyl (DPPH), metal chelating activity, azinobisethylbenzothiazolinesulphonate (ABTS), ferric reducing antioxidant power assay (FRAP), lipid peroxidation assay and ascorbic acid[11]. The richness of nutrients and bioactive compounds in SM leaves can be helpful to maintain nutrient balance as well as to combat various nutrient deficiency disorders.

The drying process is the best method to keep the nutrients and bioactive compounds intact to their maximum level in medicinal herbs. Also, reduces the cost of the final product because the weight determines the transportation as well as storage charges. Furthermore, the shelf life of the product also increased because moisture is the key factor for microorganism growth[12].Additionally, oxidation in food also causes spoilage and directly affects the nutrients, quality, taste, flavor, and texture of food [13].Dehydration of the leaves can be done by using various methods as described in peppermint leaves[14]and *Stevia rebaudiana* leaves[15]. It is very important to draw attention to the effect of

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different drying techniques on the nutrition,and antioxidant activity of leaves[16]. In this regard, the protein and Ca content of the Stinging nettle was higher in sun drying as compared with microwave drying[17]. In *Moringa oleifera*, shade drying obtained larger amounts of nutrients and minerals as compared with the sun and oven drying[18]. In *Ocimumbasilicum* leaves, antioxidant activity was highest in freeze-dried leaves as compared with fresh ones [19].

Clearly, it is indicated that there is a discrepancy in the extraction of nutrientsand antioxidant activity in medicinal herbs by using various drying methods[20]. Furthermore, as far as the authors know, there is no research conducted on the influence of various drying methods on the proximateand antioxidant activity of SM leaves. Therefore, the aim of the current study was to assess how different drying methods, sun drying (SD), shadow drying (SHD), and oven drying (OD) affect the proximate compositionand antioxidant activity of SM leaves.

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2. MATERIALS AND METHODS

2.1Chemicals

The analytical grade chemicals were used for the study. The list of the chemicals used was Mayer's reagents (MHS16), Dragondroff's reagent (44578), bromocresol green (114359), and potassium thiocyanate (207799)were collected from Sigma-Aldrich (St. Louis, MO, USA). 2,2- diphenyl-1-picrylhydrazyl (DPPH) (95% purity), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) (98% purity), 2,4,6-tri(2-pyridyl)-s-triazine ferric chloride hexahydrate, copper sulphate, potassium sulphate, sodium hydroxide, sulphuric acid, boric acid (MB007), ethanol, methyl red, potassium permanganate, potassium persulfate, hydrogen chloride, ferrous ammonium sulphate, ammonium sulphate, ammonium oxalate, sodium oxalate, ammonium hydroxide, oxalic acid, chloroform, and methanol were obtained from SRL (Mumbai, India).

2.2 Collection of Samples

The SM fresh leaves (FL) were collected from Patanjali Herbal Botanical Garden, Uttarakhand, India. The leaves werewashed manually under running tap water to remove all the dust, dirt, mud, and damaged ones. The SM leaves are separated into four batches. One batch was considered as a FLwithin 24 h of harvesting time.

2.2.1 Methods of Drying

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The left three batches were dried under different drying methods. The drying conditions employed in these methods were selected after a number of trials so as to achieve a percentage of moisture <20% by using less time for the drying process.

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2.2.2 Sun-drying (SD)

20 gof SM leaves were wrapped in a muslin cloth and placed on a round plate in sunlight for 2 days at (32±4°C). The wrapped cloth turns occasionally whichallows even drying. Also,the cloth helps to protect the leaves from unwanted dust particles and insects from the environment. After that kept the leaves in a cellophane bag.

2.2.3 Shadow-drying (SHD)

20 gof SM leaves were wrapped in a thin paper sheet and placed on a round plate in a well-ventilated room for 2-3 days at(28±2)°C temperature. Also, natural airflow in the room was used to dry the leaves, which became crispy and brittle to the touch. Kept the leaves in a cellophane bag for further analysis.

2.2.4 Oven-drying (OD)

20 gof SM leaves were spread on a glass tray and placed in an oven for about 2 h at 80 °C temperature. Then after kept the dried leaves in a cellophane bag for further analysis.

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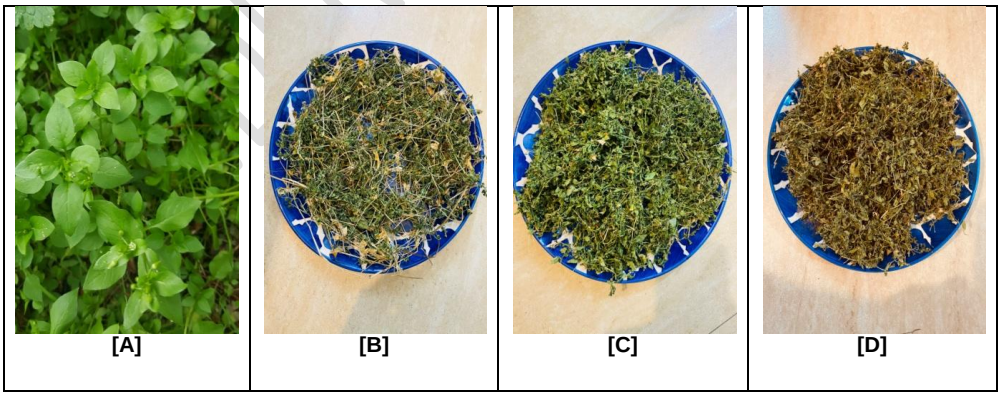

Fig. 1. Leaves of *Stellaria media* [A] Fresh leaves, [B] Sun-drying, [C] Shadow-drying, [D] Oven-drying

2.3 Extraction of Samples

Sample extraction was done according to the standard procedure given by [21]. 1 g FL and dried samples (SD, SHD, and OD) and 100 ml of 80% ethanol was taken into conical flask and covered with the aluminium foil. At 28 °C temperature and 160 rpm, the above solution was shaken in an incubator shaker (Lab Companion, SI 600R) for 26 h. Then after centrifuge (Kubota Corporation, model 4000) for 40 min at 2500 rpm to obtained a crystal-clear solution of extract. The similar procedure was repeated for the methanol (80% v/v) and distilled water.

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Comment [B16]: The similar procedure was repeated by using methanol (80% v/v) and distilled water

2.3.1 Proximate Composition

The proximate analysis of SM FL and dried leaves was done according to the procedure given by Association of Official Analytical Chemists (AOAC)[22]. The moisture content was analysed by drying the sample in hot air oven at 105 °C until the constant weight reading was achieved. Ash was determined in 12 h at 550 °C by using muffle furnace. The protein content was analysed by using micro Kjeldahl's distillation method[23]. The crude fiber of the sample was obtained by the method[24]. In addition, minerals such as calcium (Ca) and iron (Fe) were analysed by Atomic Absorption Spectroscopic standard method by using a BUCK Scientific 200A apparatus [25].

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2.3.2 Determination ofTotal Flavonoid Content (TFC)

The TFC was determined by using the previously described method with slight modification by [26]. Approximately, 1 ml of diluted extract sample mixed well in 4 ml of distilled water. Then after, 0.3 ml of (5% w/v) $NaNO_2$ was added. Then, 0.3 mL of (10% w/v) $AlCl_3$ was added after 5 min. About 2 ml of 1 M solution of NaOH was added after 6 min. Simultaneously, with the addition of 2.4 ml of distilled water, the volume reached10 ml. The mixture was shaken forcefully, and read the absorbance at 510 nm by using a UV-visible spectrophotometer (Shimadzu, UVmini-1240). With the help of a standard quercetin solution, a calibration curve was formed. On the basis of fresh weight, the result was expressed inmg quercetin/g sample.

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2.3.3 Determination of 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Free-Radical Scavenging activity

DPPH was performed by using the method explained by [27] with some modifications. Fresh stock DPPH solution was made by mixing methanol in DPPH solution at 100 µmole/L concentration. From solvent extraction, a sample extract of 1 ml was added to 6 ml of DPPH solution. The obtained mixture was vortexed for 1 min and left the mixture for 30 min in a dark place. Absorbance was

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measured by using UV-Vis Spectrophotometer (Shimadzu, UVmini-1240) at 517 nm wavelength against a methanol blank. The obtained result was calculated by using % DPPH free radical scavenging activity by using the standard formula explained below.

% Inhibition = [($A_{control}$ – A_{sample}) / $A_{control}$] x 100 %

2.3.4 Determination of Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay was determined by using [28]. Fresh FRAP reagent was prepared by mixing sodium acetate buffer 300 mM (pH 3.6) with 2,4,6-tris (1-pyridyl)-5- triazine (TPTZ) solution 10 mM in HCl40 mM and $FeCl_3.6H_2O$ 20 mM in 10:1:1 volume ratio. After that, a perfectly diluted sample in small amounts with the volume of 200 µl was added to FRAP reagent 3 ml. In the water bath, incubated the mixture for 30 min at 37 °C. The absorbance was read by using a UV-Vis spectrophotometer (Shimadzu, UVmini-1240) against the blank at 593 nm. Ferrous sulphate aqueous solution $FeSO_4.7H_2O$ (200, 400, 600, 800 µM) was used to prepare a calibration curve. FRAP values were expressed on a fresh weight basis as micromoles of ferrous equivalent Fe (II) per gram of sample.

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2.4 Statistical Analysis

The data were expressed as a mean ± standard deviation. The data analysis was performed by using analysis of variance (ANOVA) GraphPad software (GraphPad Prism 8.0.2). For intergroup comparisons, Turkey's post hoc companions were used. Differences with ($P<0.05$) were considered significant as determined by the least significant difference (LSD).

3. RESULTS

3.1 Proximate Composition

Table 1 and Fig. 2 showthe proximate composition of FL, SD, SHD, and OD leaves of SM. The parameters determined were moisture, ash, protein, crude fiber, Ca, and Fe which are notably affected by drying methods. The moisture content was higher in FL as compared to the SD ($P<0.05$), SHD ($P<0.05$), and OD ($P<0.05$) leaves respectively. Ash content which was a determinant of mineral content in leaves was higher in OD than in FL ($P<0.05$), and those dried with SD ($P<0.05$), and SHD ($P<0.05$). Protein and crude fiber were higher in dried samples as compared with the FL value significantly ($P<0.05$). Ca and Fe were higher in SD leaves as compared with FL, SHD, and OD leaves ($P<0.05$) respectively.

Samples	FL	SD	SHD	OD

Moisture (g/100g)	86.6±0.22****	7.9±0.22****	6.2±0.03****	4.4±0.31****
Ash (g/100g)	2.7±0.31ns	3.8±1.5ns	2.5±0.53ns	5.5±0.06ns
Protein (g/100g)	2.4±0.03****	10.5±0.19****	9.2±0.24****	10.3±0.35****
Crude fiber (g/100g)	6.5±0.07****	10.3±0.11****	9.4±0.15****	12.7±0.43****
Calcium (mg/100g)	160.4±0.14****	1780.4±0.43****	1470.6±0.23****	1644.4±0.36****
Iron (mg/100g)	7.2±0.00****	12.2±0.11****	10.1±0.07****	11.2±0.09****

Table. 1 Proximate composition in leaves of *Stellaria media*.

Values are means ± SD (n=3). Values are followed by **** which are significantly difference at (P<0.05). Values expressed as ns are not significantly differences at (p>0.05), where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying).

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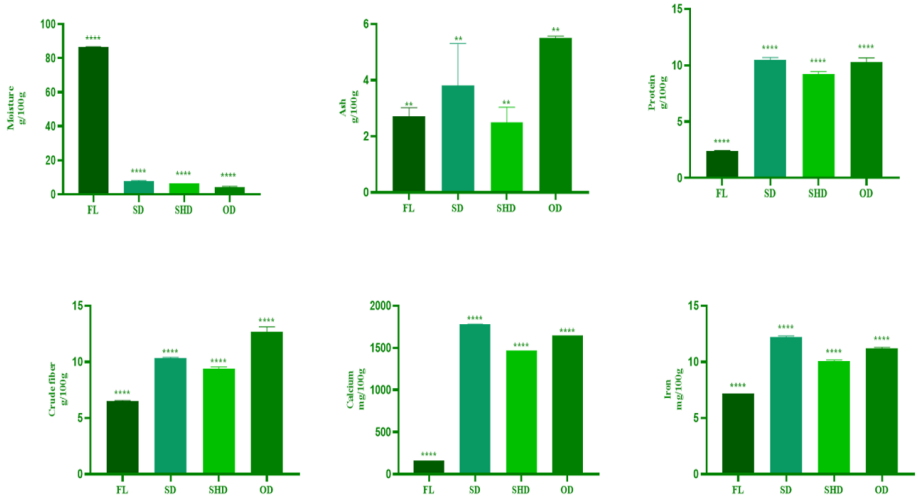

Fig. 2.Proximate composition (A) Moisture, (B) Ash, (C) Protein, (D) Crude fiber, (E) Calcium, and (F) Iron in leaves of *Stellaria media*.Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying). Values are followed by ** which are significantly difference at (P<0.05).**

3.2 Total Flavonoid Content

Table 2 and Fig.3 show that the TFC was the highest recorded in the OD samples in methanol extract (652.0±0.56 mg quercetin/g), OD samples with ethanol extract (586.0±0.71mg quercetin/g), SD sample with methanol extract (521.4±0.21mg quercetin/g), SHD samples with methanol extract (490.0±0.12mg quercetin/g), FL samples with methanol extract (456.1±0.44mg quercetin/g), SD samples with ethanol extract (413.1±0.22mg quercetin/g), OD samples with water extract (398.0±0.52mg quercetin/g), FL samples with ethanol extract (382.0±0.51 mg quercetin/g), SD samples with water extract (345.0±0.33 mg quercetin/g), SHD samples with ethanol extract (321.2±0.11mg quercetin/g), SHD samples with water extract (178.0±0.42mg quercetin/g), and FL samples with water extract (120.0±0.11mg quercetin/g) respectively.

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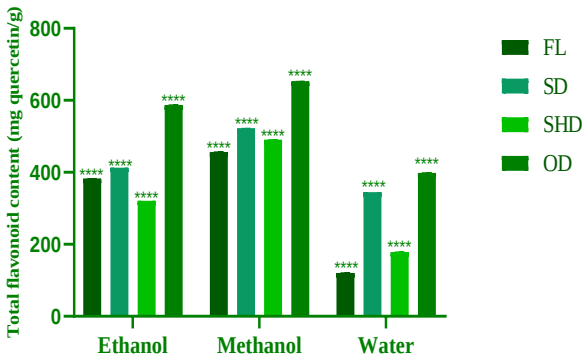
Table. 2 Total flavonoid content(mg quercetin/g) of the different extraction solvent from *Stellaria media* by using different drying methods.

Solvent types	FL	SD	SHD	OD
Ethanol	382.0±0.51****	413.1±0.22****	321.2±0.11****	586.0±0.71****
Methanol	456.1±0.44****	521.4±0.21****	490.0±0.12****	652.0±0.56****
Water	120.0±0.11****	345.0±0.33****	178.0±0.42****	398.0±0.52****

Values are means ± SD (n=3).Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying). Values followed by ****indicate significant differences at (P<0.05) by using different drying methods.



Solvent types	FL	SD	SHD	OD
Ethanol	5.3±0.13****	46.7±0.25****	59.3±0.21****	78.5±0.11****

Fig. 3.Total flavonoid content(mg quercetin/g) in leaves of *Stellaria media* by using different drying methods with different extraction solvent. Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying).Values followed by ** indicate significant differences at (P<0.05).**

3.3 Determination of Antioxidant Activity (DPPH and FRAP)

The antioxidant activity in DPPH and FRAP showed a wide range of variation among the fresh or drying samples as well as solvent types. Furthermore, the inhibition percentage in DPPH ranged from 4.8±0.17 in FL in water extract to 95.8±0.21in OD samples in methanol extract in Table 3 and Fig.4. The value of FRAP ranged from 9.0±0.42 µmolFeII/gin FL in water extract to 9277.0±0.33µmolFeII/g in OD samples in methanol extract in Table 4 and Fig.5. In both activity, results showed the similar trend of result in which highest activity showed in OD samples in methanol extract and lowest activity in FL samples in the water extract. Also, a significant difference(P<0.05) was also observed in all the samples in three different solvent types.

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Solvent types	FL	SD	SHD	OD
Methanol	10.9±0.33****	69.9±0.11****	77.8±0.23****	95.8±0.21****
Water	4.8±0.17****	37.0±0.22****	42.6±0.23****	54.9±0.31****
Ethanol	19.6±0.22	88.9±0.22	446.0±0.11	765.6±0.22

Table. 3DPPH inhibition percentage (%) of the different extraction solvent from *Stellaria media* by using different drying methods

Values are means ± SD (n=3).Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying). Values followed by **** indicate significant differences at (P<0.05) by using different drying methods.

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Fig. 4.DPPH inhibition percentage (%) in leaves of *Stellaria media* by using different drying methods with different extraction solvent. Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying).Values followed by ** indicate significantdifferences at (P<0.05).**

Table 4. Ferric reducing power (µmolFeII/g) of the different extraction solvent from *Stellaria media* by using different drying methods.

Methanol	12.0±0.34****	44.0±0.54****	777.2±0.33****	9277.0±0.33****
Water	9.0±0.42****	18.0±0.11****	365.0±0.33****	433.0±0.00****

Values are means ± SD (n=3).Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow- drying), and OD (Oven-drying). Values followed by **** indicate significant differences at (P<0.05) by using different drying methods

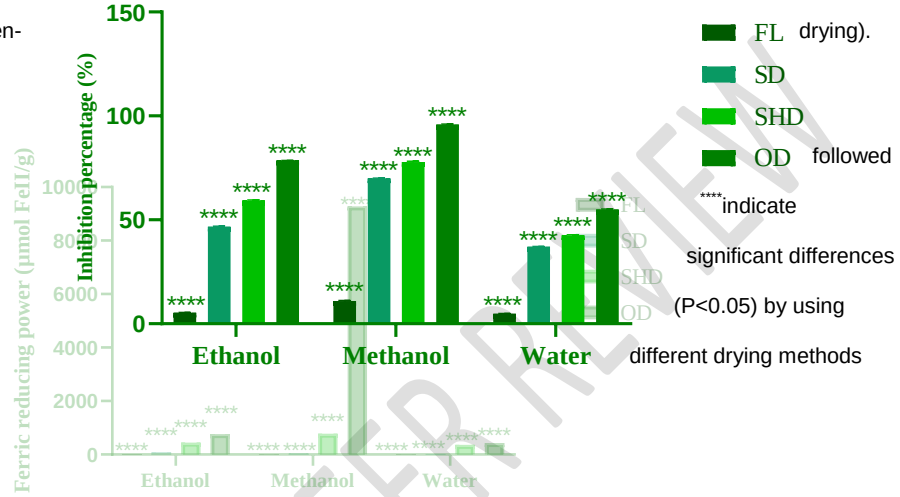

Fig. 5.Ferric reducing power (µmolFeII/g) in leaves of *Stellaria media* by using different drying methods with different extraction solvent. Where FL (Fresh leaves), SD (Sun-drying), SHD (Shadow-drying), and OD (Oven-drying).Values followed by ** indicate significant differences at (P<0.05).**

4. DISCUSSION

The most critical parameter that was analyzed during the storage of dried medicinal plant leaves is its moisture content [29]. One study reported that low moisture content in raw material increases the shelf life of the developed food products [30]. The ash content presents the availability of mineral

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content in the developed food from the raw material [31]. The study conducted by [17] in Stinging nettle leaves showed higher content of protein was present in SD leaves as compared with SHD, the study supports the result of the present investigation. The study conducted in basil leaves reported that crude fiber in basil leaves was 10% which is nearly the value obtained by present result in dried samples [32]. Furthermore, the study [17] also confirmed that significance of drying processincreases all the more to keep its nutritional components intact to maximum. TFC showed the same trend of result as showed by DPPH and FRAP. The main reason behind similarity described by that flavonoids possess antioxidant capacity and free radical scavenging potential [33]. The other study concluded that polyphenolic compounds consist of flavonoids with benzo-γ- pyrone structure are present widely in plants. Antioxidant activity is mainly due to the presence of flavonoids in green leafy vegetables [19]. Additionally, antioxidant activity of *Ocimum sanctum* leaf and *Terminalia chebula*fruit was 92.9% and 58% respectively [34].Both of these previous results are in the range of the current study. Nonetheless, the value of percentage of inhibition varies in each study due to several factors, such as temperature, pH, duration during storage, and type of solvent used [35]. Furthermore, the result of the FRAP assay, in the previous study concluded that dried leaves with methanol extraction showed a maximum FRAP value as compared with the FL[21]. The study supports the results of the current study findings.

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5. CONCLUSIONS

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The current study complements and expand the literature knowledge of the SM plant with special emphasis was given to the proximate and antioxidant activity. Also, SM with no side effects having a number of medicinal properties. Furthermore, the current research aided the information about the proximate, minerals, and antioxidant activity were present in the leaves of SM which may be the key factor for its pharmacological activity. Leaves of SM in dried form also a good source of protein, Ca and Fe. Additionally, methanol extract with OD leaves showed highest antioxidant activity and lowest activity showed in FL with water extract.Apart from this, it may also lead to the development of an innovative food products for preventive healthcare in the competition of expensive drugs.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable

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