INTRODUCTION

Reactive oxygen species (ROS) are natural byproducts formed by cellular activity and include free radicals such as hydroxyl radicals, superoxides, and singlet oxygen. These ROS are essential for the progression of biological processes like cellular proliferation, differentiation, apoptosis and intracellular signaling (Ron, 2017; Di Meo et al., 2016) and the overproduction due to stress, lifestyles, pollution and exposure to UV...
(Ultraviolet) radiation. Excessive production of these reactive species is linked with degradation of biological components such as proteins, lipids and nucleic acids, leading to tissue injury and damage to health conditions (Zhang et al., 2016). A large number of pathological conditions, such as rheumatoid arthritis, ischemia, cancer, diabetes, metabolic disorders, are linked with oxidative and chronic stress (Chaudhary et al., 2020; and Di Meo et al., 2016). To manage increased oxidative stress in both healthy and pathological conditions, antioxidants are used more frequently and produced exogenously from outside (Plants) or endogenously through enzymes such as catalase, glutathione peroxidase and superoxide dismutase (Patekar et al., 2013). Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are dangerous to human health and show carcinogenic effects (Kahl et al., 1996). So, the research related to natural antioxidant has gained importance (Lobo et al., 2010). Polyphenols, flavonoids and essential oils are secondary metabolites derived from aromatic and medicinal plants that serve as reducing agents, free radical scavengers, inhibitors of oxidative enzymes, prevent rancidity caused by lipid oxidation as well as the onset of diseases connected with oxidative stress (Baier and Dietz, 2005; Frankel, 1995; Ghasemzadeh et al., 2011; and Chaudhary et al., 2020).

In Ayurveda, Siddha, Unani and other traditional systems, around 35000 medicinal plants are listed for their therapeutic effects (Panchal et al., 2019). Holy basil or Ocimum sanctum/Ocimum tenuiflorum is a well-known popular plant used for medicinal purposes, also known as Tulsi. This aromatic perennial plant of the Lamiaceae family is indigenous to the Indian subcontinent and listed in Charaka Samhita (an Indian traditional medicine scripture) and has two varieties: light green leaves Rama/ Lakshmi Tulsi and purple leaves or dark stem Shyama / Krishna Tulsi (Ocimum tenuiflorum). Shyama Tulsi has more antioxidant properties than Rama Tulsi (Parajuili-Bara, 2023). Mondal et al. (2009) reported that chemical constituents of both varieties are similar, whereas Upadhyay et al. (2015) and Singh et al. (2018) documented some other metabolites with higher gene expression and concentration in Shyama Tulsi. These metabolites are attributed to higher antioxidant, anticancerous, anti-inflammatory, anti-fungal, antiseptic, analgesic, antidiabetics, radioprotective and anti-stress properties (Singh and Chaudhuri, 2018; Kumar et al., 2022; Priya and Peddha, 2023).

The leaves of Ocimum contain various compounds such as eugenol, methyl eugenol, carvacrol, eugenol methyl ether, rosmarinic acid and apigenin (Gupta et al., 2002). The extraction of phytochemicals is a challenging process as the polarity of solvents affects the antioxidant potential and its compounds. A well-known approach, i.e., sequential fractionation, is used to get maximum antioxidants from herbs. This method was used by Chaudhary et al. (2020) to study the leaves of Ocimum sanctum and get maximum polyphenols and flavonoids. However, other studies are also available for the maximum extraction of polyphenols and flavonoids through sequential fractionation in Ocimum sanctum, but not reported in Rama or Shyama Tulsi (Chaudhary et al., 2020; and Ao et al., 2008). To date, no clear record of antioxidant activity of Shyama Tulsi through sequential fractionation methods is available in the literature. So, the present study aimed to study the antioxidant potential and phytochemicals of O. tenuiflorum (Shyama Tulsi) through the sequential fractionation method.

**MATERIALS AND METHODS**

**Chemicals**

DPPH (2,2-Diphenyl-1-picrylhydrazyl), Ascorbic acid, Aluminium Chloride, trichloroacetic acid (TCA), ferric chloride, Folin–Ciocalteu reagent, ABTS (2,2’-azinobis (3-ethylbenothiazolone-6-sulfonic acid), methanol, n-hexane, ethyl acetate and n-butanol were of analytical grade and procured from Merck (Mumbai, India). The ultrapure water was prepared using the Milli-Q System (Merck, Bombay, India).

**Plant material**

*Ocimum tenuiflorum* (Shyama Tulsi) seeds of genotype HS01 were procured from the Department of Genetics and Plant Breeding, College of Agriculture at Chaudhary Charan Singh Haryana Agriculture University (CCS HAU), Hisar, India. Small-sized seeds were planted in seed pots (Chaudhary et al., 2020). To accelerate germination, the soil was kept moist. After 25-30 days, emergence of seedlings took place. When germination was greater than 85%, seedlings were transferred to earthen pots. Plants were grown without pesticides for three months, and young leaves were collected, cleaned with clean water and stored for two days at room temperature. Leaves were kept for seven days at 45°C in an oven to maintain a constant dry weight (Chaudhary et al., 2020).

**Preparation of extract and fractionation**

Coarsely powdered 30 g of dry leaves were extracted thrice with 50% methanol (1g/10ml) for 24 hours at room temperature in a shaker and pooled. After passing through double-layered muslin cloth, the extract was centrifuged at 3000g for 5 min (Chaudhary et al., 2020). The crude dried extract of *O. tenuiflorum* in methanol solvent (OTM) was obtained (26.41%) after solvent evaporation at 40°C in a rotary evaporator. After that, crude extract was suspended in 60 ml of dis-
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tilled water and partitioned with n-hexane (150 ml ×3), ethyl acetate (150 ml ×3) and n-butanol (150 ml ×3) to obtain *O.tenuiflorum* hexane (OtH), *O.tenuiflorum* ethyl acetate (OtE), *O.tenuiflorum* butanol (OtB), and *O.tenuiflorum* residue (OtR) fractions (Fig 1).

Determination of total polyphenolic content

The extract aliquots were combined with 2 ml of 2% sodium carbonate to evaluate the total phenolic content of the preparations. After two minutes, 100 µl of 1 N Folin-Ciocalteau was added and absorbance was read spectrophotometrically at 750 nm after 30 min (Dua et al., 2013). Gallic acid was used to prepare a standard curve (0-100 nmoles) and phenolic content was determined as mg gallic acid equivalent (GAE).

Determination of total flavonoid content

For the estimation of flavonoid content of the sample, an aliquot of the extract was diluted to form a volume 0.5 ml; 30 µl of NaNO₂ (5%) was added and thoroughly mixed (Dua et al., 2013). After 5 min, 200 µl of NaOH (1N) was mixed and read at 510 nm. Flavonoids were reported as mg quercetin equivalents (QE), and a standard curve was prepared using quercetin (0-100 nmoles).

**In vitro** determination of antioxidant activity

2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH solution (50 ×10⁻⁵ M) was mixed in methanol with 1 ml of extracts prepared with various solvents (Brand-Williams et al., 1995). After 15 min of incubation, absorbance was observed at 517 nm ([Ab - Aa]/Ab) ×100 was used to calculate the decrease in the percentage of DPPH free radical scavenging activity where Ab was the optical density (OD) of control without extract; Aa was OD with extract in the reaction mixture. The amount of extract (IC₅₀) that scavenged 50% of free radicals was calculated.

2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation radical scavenging activity

As reported in the literature (Re et al., 1999), the cation radical Scavenging activity was determined using the solution 7mM ABTS prepared after mixing 2.45mM ammonium persulfate and left overnight in the dark to obtain cation radicals. Various extracts were mixed with an adequately diluted solution, and a reduction in absorbance at 734 nm was recorded. IC₅₀ and scavenging activities were determined.

**Ferric Metal Ion Reducing Power( FRAP)** metal ion-reducing property

The FRAP reagent (0.2 M phosphate buffer, 1% potassium ferricyanide, 0.1% ferric chloride) was prepared to determine the metal ion (Fe (III)) reducing property (Gupta and Panday 2014). The extract fraction was combined with the FRAP reagent, incubated at 50°C for 20 minutes and the absorbance at 700 nm was measured. The IC₅₀ value and antioxidant activity were calculated.

**High Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) analysis**

HR-LCMS) was performed on a Q-TOF mass spectrometer to confirm the presence of active constituents in the leaf extract of Shyama Tulsi (Anand et al., 2022). The HiP sampler, binary gradient solvent pump,
column compartment and quadrupole time of flight mass spectrometer (MS Q-TOF) with dual Agilent Jet Stream Electrospray (AJS ES) ion sources comprised the liquid chromatographic system. 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) were used as solvents. A 0.300 mL/min flow rate was used and MS (Mass Spectrometry) detection was performed in MS Q-TOF. Compounds were identified via their mass spectra and their unique mass fragmentation patterns.

Statistical analysis
The results were shown as mean ± SD. The software SPSS/Window (SPSS 16.0) was used to calculate the IC50 values. To analyze the differences in IC50 values of various fractions and the relationship between IC50, polyphenolic and flavonoid content, one-way ANOVA test and Tukey’s test (p<0.05) were used.

RESULTS AND DISCUSSION

Extraction, total polyphenolic and flavonoid content
The percentage yield from 50% methanolic leaf extract of Shyama Tulsi (O.tenuiflorum) was 26.41% (Table 1). 50% methanol extract of Shyama Tulsi leaves was subjected to differential and sequential fractionation with n-hexane, n-ethyl acetate, n-butanol and observed 0.30-6.67% yield, i.e. 1.13 to 25.25% extraction of total soluble components in methanol extract. The maximum amount of soluble components, 25.25% was extracted with n-butanol fraction, whereas 8.85% in ethyl acetate fraction and very small amount of 0.30% was extracted with n-hexane. This showed that a very small percentage of methanol soluble components were non-polar and were extracted with n-hexane. Similarly, Borah et al. (2018); and Chaudhary et al. (2020) reported 8% and 19.27% extraction yield in methanolic extract of O.sanctum leaves, respectively, lower than the current study.

Total polyphenolic content (TPC) with different fractions was determined by using Folin-ciocalteau and measured as Gallic acid equivalent (GAE), with values ranging from butanol to hexane fraction, i.e. 288.82±0.45 mg/g to 85.60±0.19 mg/g gallic acid equivalent respectively (Table 1). Amin et al. (2013) illustrated TPC in the ethanolic extract of O. sanctum leaves was 279.05±0.73 mg/g of gallic acid equivalent and Wangcharoen et al. (2007) documented TPC in the ethanolic extract of Shyama and Rama Tulsi was 19.46±1.97 to 12.60±1.02 mg GAE/g respectively. While Ishwar Pathak and Muna Niraula (2019) stated TPC content in methanol extract 80.21 ± 0.89 mg GAE/g. Similarly, TPC with methanolic extract of O. tenuiflorum was 114.34 mg GAE/g, as reported by Vastrad et al. (2015). Chaudhary et al. (2020) revealed TPC in OtM was 87.13 ± 4.6 mg GAE/g. In the present study, the TPC of methanolic extract in O.sanctum were 863.63±0.17 mg GAE/g, which was higher than reported literature studies while TPC content in O.sanctum with butanol solvent i.e. OsB fraction was 212.26 ± 6.3 mg GAE/g, while in the present study, it was 288.82±0.45 GAE/g with butanol fraction. Ishwar Pathak and Muna Niraula (2019); and Chaudhary et al. (2020) reported TPC content with hexane fraction of O.sanctum was 60.55 ± 0.25, 52.68 ± 1.8 mg GAE/g respectively, which was lower than the present study. The present findings showed higher TPC in Shyama Tulsi compared to previous studies and varied in the following order: OtM>OtB>OtE>OtH>OtR.

Total flavonoid content (TFC) was measured as quercetin equivalents varies from methanol 310.16±0.25 to 56.89±0.18 mg QE/g hexane fraction (Table 1). Results indicated that TFC was highest in methanol extract and lowest in residual fraction. Vastrad et al. (2015) observed that the TFC content of methanolic extract of O.tenuiflorum was 96.34 mg QE/g of dry extract. Pathak and Niraula (2019); and Chaudhary et al., (2020) reported TFC content with methanol fraction was 67.11 ± 0.43 and 221.97 ± 4.6 mg QE/g, while in hexane fraction was 16.91 ± 0.56, 32.86 ± 2.2 mg QE/g of dry extract respectively. In present study, TFC content with all fractions was greater than previously reported by Chaudhary et al. (2020) in O.sanctum. Overall, the data depicted that TFC content, TPC, and % yield were higher when compared with previous studies, and maximum polyphenols and flavonoids were extracted in butanol fraction followed by ethyl acetate.

Table 1. Total polyphenols, flavonoid and extraction yield of methanol and soluble components of Shyama Tulsi

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Phenolic mg GAE/g</th>
<th>Flavonoid mg QE/g</th>
<th>Extraction yield (%)</th>
<th>% Extraction of soluble components from OtM</th>
</tr>
</thead>
<tbody>
<tr>
<td>OtM</td>
<td>863.63±0.17a</td>
<td>310.16±0.25a</td>
<td>26.41±0.56a</td>
<td>100</td>
</tr>
<tr>
<td>OtH</td>
<td>85.60±0.19d</td>
<td>56.89±0.18d</td>
<td>0.30 ±0.12a</td>
<td>1.13</td>
</tr>
<tr>
<td>OtE</td>
<td>269.98±0.40c</td>
<td>91.22±0.26c</td>
<td>2.26±0.46b</td>
<td>8.85</td>
</tr>
<tr>
<td>OtB</td>
<td>288.82±0.45b</td>
<td>101.11±0.24b</td>
<td>6.67±0.54c</td>
<td>25.25</td>
</tr>
<tr>
<td>OtR</td>
<td>75.25±0.49b</td>
<td>26.96±0.10a</td>
<td>14.15±0.60d</td>
<td>53.57</td>
</tr>
</tbody>
</table>

Each value in the table is represented as mean ±SD (n=3). Value in the same column followed by a different later (a-e) are significantly different (p<0.05). OtM (50% methanol), OtH (n-hexane fraction), OtE (Ethyl acetate fraction), OtB (n-butanol fraction), OtR (Residue fraction)
fraction. Compared to water and other solvents, methanol is a better solvent for extracting polar polyphenols and flavonoids (Kchaou et al., 2013). Leaf extracts of Ocimum tenuiflorum in hexane, acetone and methanol had been examined (Naik et al., 2015) but differential extraction was not reported previously (Rabeta et al., 2013). However, Chaudhary et al., (2020) showed differential extraction and concentration of polyphenols and flavonoids from Ocimum sanctum leaves. The activity of polyphenols in various solvents may also be influenced by varied amounts and composition of phytochemicals (Agarwal et al., 2017; and Saeed et al., 2012). However, Shyama Tulsi has more antioxidant potential than green leaves (Parajuli-Bara 2023). In addition, a different assay was performed to determine the antioxidant potential of Shyama Tulsi extract and their ability to scavenge various types of free radicals or decrease in metal ions property.

In vitro antioxidant activity

To evaluate antioxidant potential of Shyama Tulsi leaf extract and its fractions in various solvents (50% methanol, n-hexane, ethyl acetate and n-butanol), three distinct methods i.e. DPPH, ABTS and FRAP were used. The results were shown (Fig. 2 a-c). The IC50 values were calculated to compare its effectiveness with the standard and among themselves (Table 2).

DPPH radical scavenging activity

The analysis of antioxidant activity of different fractions through DPPH free radical scavenging potential (Fig 2a) was in following order: OtB>OtE>OtM>OtH>OtR. At a concentration of 4.8±0.23 µg/ml butanol fraction exhibits 50% DPPH scavenging activity (Table 2) lower than ethyl acetate and methanol fraction i.e 6.85±0.51 and 8.5±0.24 µg/ml respectively. While IC50 value with standard ascorbic acid under identical condition was 4.2±0.24 µg/ml. The result indicated that n-butanol fraction has more antioxidant potential compared to other fractions. IC50 value with ethyl acetate fraction in O. sanctum reported by Chaudhary et al., 2020 was higher than current study. This may be due to more essential oils in our extract, which contributes to decreased IC50 value of DPPH assay. Fadila et al. (2017; and Chaudhary et al., 2020) observed DPPH free radical scavenging activity in methanol fraction with an IC50 value 12 µg/mL, 10 µg/mL, respectively, while Toppo et al. (2019) documented IC50 value 40.43µg/ml with methanolic leaf extract of O.sanctum. Parajuli-Bara (2023) reported IC50 value for DPPH free radical scavenging activity in ethanolic extract of Shyama Tulsi was 8.83µg/ml while in green leaves 10.41µg/ml. In the present study IC50 value with methanolic extract was 8.5 µg/ml lower than previously reported (Fadila et al., 2017; and Chaudhary et al., 2020). Chaudhary et al., (2020) stated OsH exhibits higher IC50 value compared to the current observation. All the previously reported studies showed higher IC50 value than current study which depict higher antioxidant potential in Shyama Tulsi. This may be due to more active constituents that could transmit hydrogen atom to free radical and lower the IC50 value. Both n-butanol and ethyl acetate were able to extract active antioxidants from holy basil leaves and they both had a significant amount of free radical scavenging capacity, however n-butanol exhibited better efficiency. Polyphenols extracted in hexane fraction showed higher IC50 value and was found ineffective in scavenging DPPH free radicals, which may be due to the formation of active part complexed with non-polar components (Chaudhary et al., 2020).

Table 2. Radical scavenging activities of Shyama Tulsi fractions

<table>
<thead>
<tr>
<th>Extract/fraction</th>
<th>DPPH assay IC50 (µg/ml)</th>
<th>ABTS assay IC50 (µg/ml)</th>
<th>FRAP assay IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Ascorbic acid</td>
<td>4.2 ±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ±0.0002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OtM</td>
<td>8.5±0.24&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.6 ±0.34&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>22.5±0.002&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>OtH</td>
<td>12±0.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.2±0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48 ±0.003&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>OtE</td>
<td>6.85±0.51&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.8±0.46&lt;sup&gt;e&lt;/sup&gt;</td>
<td>20 ±0.004&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>OtB</td>
<td>4.8±0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4±0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.5±0.004&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>OtR</td>
<td>21±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.5±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value in the table is represented as mean ± SD (n = 3). Values in the same column followed by a different letter (a-e) are significantly different (p< 0.05). OtM (50% methanol), OtH (n-hexane fraction), OtE (Ethyl acetate fraction), OtB (n-butanol fraction).OtR (Residue fraction)
ABTS radical scavenging activity
The organic cation radical activity of an extract was measured by ABTS assay. Results of the present study showed OtB fraction has higher ABTS radical scavenging activity than all other fractions (Fig. 2b). The ABTS cation radical scavenging activity was in order as OtB>>OtE>OtM>OtH>OtR. OtB fraction has a greater ability to scavenge cation radicals than ascorbic acid and a lower value of IC$_{50}$ indicated the presence of well-organized organic cation scavengers (Table 2). OtE fraction had more antioxidant potential than leaves of O.sanctum reported by Chaudhary et al., 2020 and may be due to some active constituents in our extract. While Toppo et al. (2019); Basak et al. (2014); Chaudhary et al. (2020) illustrated higher IC$_{50}$ values with methanolic extracts of O.sanctum as 53.5µg/ml, 131µg/ml, 9.2 µg/ml respectively. The present study showed that Shyama Tulsi extract had more ability to scavenge cation radicals than the reported literature.

FRAP metal ion reducing property
FRAP assay was used to detect total antioxidant ability of any extract. In the present study, metal ion reducing property followed the OtB>OtE>OtM>OtH>OtR trend. IC$_{50}$ value of OtB fraction was 14.5±0.004 µg/ml much lower than hexane (48±0.003µg/ml), and methanol fraction (22.5±0.002 µg/ml) (Table 2; Fig.2c). OtB and OtE showed a linear increase in activity upto 20±0.004 µg/ml, while in another fraction increased upto 150±0.002 µg/ml (Fig.2c). The present study showed OtB and OtE fractions had lower IC$_{50}$ values indicated presence of powerful reducing agents. Agarwal et al. (2017) stated Ferric reducing activities i.e. 77.05±2.67 in ethyl acetate extract, while in butanol fraction, phenolic and flavonoid compounds may efficiently remove free radicals or exchange H+ quickly directly, contributing to the antioxidant property (Renen et al., 2010). Polyphenols influence antioxidant properties by their π electron in the benzene ring, while flavonoids impart due to the

Table 3. Correlation between phenolic and flavonoid content of Shyama Tulsi and the IC$_{50}$ values of antioxidant activities

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>Phenolics Correlation (r)</th>
<th>Flavonoid Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ of DPPH radical scavenging ability</td>
<td>0.879</td>
<td>0.846</td>
</tr>
<tr>
<td>IC$_{50}$ of ABTS radical scavenging ability</td>
<td>0.801</td>
<td>0.914</td>
</tr>
<tr>
<td>IC$_{50}$ of FRAP ion reducing capacity</td>
<td>0.967</td>
<td>0.824</td>
</tr>
</tbody>
</table>

Each value in the table is represented as mean ± SD (n=3)
keto group at C4 or hydroxyl groups at rings A and C (Ali and Neda 2011).

*In vivo* and *in vitro* studies, the beneficial properties of polyphenols and flavonoids are documented. It is reported that polyphenols restrain mutagenesis and carcinogenesis in humans (Tewari *et al.*, 2015). While *in vitro*, flavonoids inhibited lipid peroxidation (Catherine Rice-Evans 2001), oxidation of low-density lipoproteins (LDL) and prevent cellular damage associated with oxidative stress (Gate *et al.*, 1999; Pacheco *et al.*, 2009 and Pisoschi *et al.*, 2015). Further research is going on to study the protective behaviour of different polyphenols in various diseases caused by oxidative stress.

**Phytochemical analysis**

Polyphenols are the natural benefits of human beings, and there is a continuous need to explore sources of polyphenols. To date, no study has reported the extraction of polyphenols with the fractionation method in Shyama Tulsi. To analyze the polyphenols and flavonoids in detail in the best extracts of the present study, OIE or OtB fractions were subjected to HR-LCMS (Fig. 3). Hexane fraction was not proceeded for HR-LCMS due to its lower antioxidant potential. In the present study, total 26 polyphenols (Table 4) and 18 flavonoids were identified i.e. 6-C-Galactosylluteolin, Quercitrin, Isorientin, Kaempferol 3-glucuronide, Jaceidin, Genis-
Moreover, these polyphenols add to antioxidant activity (Uma Devi et al., 2014). Further, these polyphenols and flavonoid compounds identified in ethyl acetate and butanol fraction as 6-C-Galactosylluteolin, Quercitrin, Jaceidin, Myricetin 7-Rhamnoside, Butin, 2”-O-trans-p-Coumaroylastragalin, Apigenin 4’-O-glucoside, Nicotiflorin, Gallocatechin, Galangin, Baicalin, Diosmetin 7-O-beta-D-glucuronopyranoside shown in (Fig. 3). A numbers of active polyphenolics constituents such as quercetin, orientin, luteolin, apigenin, caffeic acid, chlorogenic acid, kaempferol and genistein has been reported by Chaudhary et al. (2020); Baliga et al. (2013); Upadhayay et al. (2015); Ullah et al. (2022); and Mousavi et al. (2018). But in the present study, in addition to the previously identified compounds were some other active polyphenolics, flavonoid compounds identified in ethyl acetate and butanol fraction as 6-C-Galactosylluteolin, Quercitrin, Jaceidin, Myricetin 7-Rhamnoside, Butin, 2”-O-trans-p-Coumaroylastragalin, Apigenin 4’-O-glucoside, Nicotiflorin, Gallocatechin, Galangin, Baicalin. These results justified the higher antioxidant activity of Shyama Tulsi as compared to previously reported studies (Chaudhary et al., 2020). Many biological properties of various flavonoids are reported as antibacterial, antiviral, anti-inflammatory, anticancer, antiallergic activities, protection of lipids against oxidative stress, radioprotective and enhanced DNA repair in addition to antioxidant activity (Uma Devi et al., 1999; Yao et al., 2004; Montoro et al., 2005; Shashank and Abhay, 2013; Satyamitra et al., 2014). Moreover, preclinical research demonstrated that Tulsi and its phytochemicals (Oleanolic acid, Ursolic acid, Apigenin, Rosmarinic acid, β-caryophyllene, Eugenol, Linalool, Luteolin, Myrelenol, Carnosic acid and β-sitosterol) increased antioxidant activity, altered gene expression, caused apoptosis, inhibited angiogenesis, metastasis, suppress chemically induced skin, liver, oral and lung cancer (Naik et al., 2015; and Baliga et al., 2013). Further, these polyphenols and flavonoids can be studied for their beneficial effect in various diseases.

**Conclusion**

Shyama Tulsi (*O. tenuiflorum*) leaves sequentially extracted with 50% methanol, hexane, ethyl acetate, and butanol were evaluated for their antioxidant properties or phytochemicals. The present study revealed higher phenolic and flavonoid content, indicating the natural antioxidant nature of Shyama Tulsi, signifying its medicinal importance and that it may be useful in protection against oxidative stress. The higher antioxidant potential may be due to the polyphenolic compounds such as Quercitrin, 6-C-Galactosylluteolin, Nicotiflorin, Apigenin 4’-O-glucoside, caffeic acid, Genistin, Myricitrin detected in the present study by HR-LCMS. Due to this medicinal herb’s higher antioxidant potential and diverse polyphenols, it can also be used as a substitute for synthesizing new medicines.

**Table 4. Identification of polyphenolic compound in Shyama Tulsi**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>MW</th>
<th>Compound detected</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTE</td>
<td>594</td>
<td>Kaempferol 4’-glucoside 7- rhamnoside</td>
<td>C_{27}H_{30}O_{15}</td>
</tr>
<tr>
<td></td>
<td>462</td>
<td>Kaempferol 3-glucuronide, Luteolin 7-O-glucuronide</td>
<td>C_{21}H_{18}O_{12}</td>
</tr>
<tr>
<td></td>
<td>448</td>
<td>isoorientin,6-C-galactosylluteolin, Quercitrin</td>
<td>C_{21}H_{26}O_{11}</td>
</tr>
<tr>
<td></td>
<td>432</td>
<td>Genistin, Apigenin 4’-O-glucoside</td>
<td>C_{21}H_{20}O_{10}</td>
</tr>
<tr>
<td></td>
<td>464</td>
<td>Myricitrin, Myricetin 7-rhamnoside</td>
<td>C_{21}H_{24}O_{12}</td>
</tr>
<tr>
<td></td>
<td>288</td>
<td>2-(4-Methyl-1,3-pentadienyl) anthraquinone</td>
<td>C_{20}H_{18}O_{2}</td>
</tr>
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<td></td>
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<td>Butin</td>
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Conflict of interests

The authors declare that they have no conflict of interest.

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