Comparative analysis of bioactive compounds and antioxidant capacity of selected plants as phytogenic feed additives for poultry nutrition

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ABSTRACT

The paper investigated the phytochemical content and antioxidant activity of poplar buds (Populus nigra L.), turmeric (Curcuma longa) and ginger (Zingiber officinale) extracts. Proximate composition, total polyphenols, ß-carotene, vitamin E, minerals and antioxidant capacity were tested for all selected plants. The proximate analysis of selected plants showed that poplar buds had a higher content of crude protein, fat and fiber than turmeric and ginger. The extract of poplar buds had a significantly higher content of total polyphenols (87.20 mg of gallic acid/g) and vitamin E (210.53 µg/g) than in the extract of turmeric and ginger. The polyphenol content was found to be in the order: poplar buds> turmeric> ginger. The beta carotene content was significantly higher in poplar buds (2.99 µg/g) than in turmeric (1.67 µg/g). Regarding mineral content, turmeric showed the highest content in iron and copper; ginger showed the highest content in manganese and poplar buds had the highest content in zinc. Moreover, poplar buds possessed the highest antioxidant capacity both on vitamin C and E calibration curve, evidencing the rich content in such antioxidants. Therefore, based on the valuable nutritional composition confirmed through this study, poplar buds, turmeric and ginger can be recommended as phytogenic feed additives for poultry nutrition.

Keywords: antioxidant capacity, bioactive compounds, phytogenic feed additives, poultry nutrition.

INTRODUCTION

Nowadays, there are an increasing interest in discovering novel natural sources of nutrients as alternatives to synthetic pharmaceuticals to be included in animal nutrition. Discovering alternative and readily available feed
additives is essential to protect the poultry industry, particularly in developing countries (Ayalew et al., 2022). In the context of promoting a safe diet and high quality food, the use of these feed additives as natural sources of nutrients in animal diet is a promising solution to achieve the goal by ensuring the health and welfare of animals, but also the safety of animal products (Arain et al., 2022).

The term of phytogenic feed additives are commonly defined herbs or spices which were gradually gaining popularity as supplements into animal diets to promote health and performance (Criste et al., 2017). A great number of botanical products were studied for their potential to be included as phytogenic feed additives in poultry diets including fenugreek (Khalili Samani et al., 2020), oregano leaves and oil (Vlaicu et al., 2022), artemisia leaves (Saracila et al., 2018; Panaite et al., 2019), willow bark (Saracila et al., 2019; Panaite et al., 2020) and also there are many available to be studied. Leaves, barks, buds, seeds, and flowers are among most popular parts of a botanical product that contained bioactive nutrients including phenols, vitamins, minerals, essential oils, etc. These bioactive compounds have confirmed to possess health promoting effect including antibacterial, antioxidant, antiinflammatory effect in poultry and may enhance their production and welfare, obtaining high quality products (El-Sabrout et al., 2023). Literature findings showed that phytogenic feed additives show greater modes of action in animal nutrition compared to synthetic, nature-identical substances (El-Sabrout et al., 2023).

Since ancient times, extracts from buds or barks of different trees has been widely used in traditional medicine (Radovanović et al., 2023). Poplar buds are obtained from Populus species which together with Salix species belong to the family of Salicaceae. In Romania Populus species are widespread and grow through wet meadows and depressions, but can be also found in the plains (Kis et al., 2020). In Europe, the first writings about poplar buds were in John Gerard’s book (1597), which described the use of the buds as a medicine against inflammation. Although it was initially studied for its anti-inflammatory properties, recent evidence showed that poplar poplar buds possess due to the significant amounts of flavonoids phenolic compounds, terpenoids, and more than 48 phytocompounds in the essential oils (Kis et al., 2020).

Turmeric (Curcuma longa L.) belongs to Zingiberaceae family and is an herbaceous plant cultivated for their rhizomes in Asian countries and nowadays, is consumed worldwide as a spice and seasoning (Das, 2016). In the traditional medicine, turmeric has a long history for the prevention and treatment of different diseases, due to the antioxidant, antibacterial activity (Bomdyal et al., 2017). These functional properties are due to the major components of turmeric, curcuminoids possessing radical scavenging activity
(Na et al., 2013). The typical curcuminoi
ds include curcumin, demethoxycurcumin (DMC) and bis-demethoxycurcumin (Singh et al., 2022).

Another representant belonging to Zingiberaceae family is ginger (Gingiber officinale). Ginger rhizome initially was consumed in traditional medicine as herb or spice, but in last years due to their potent antioxidant properties, attracted great interest also in Europe (Mustafa et al, 2023). Among their bioactive compounds are phenolic compounds (gingerdial, gingerol, gerdione and shogaloa), flavonoids, Fe, Mg, Ca, vitamin C, sesquiterpenes, paradols (Shahrajabian et al., 2019). Among biogical efects of ginger are anti-inflammatory, anti-tumor activities, anti-pyretic, anti-platelet, anti-hyperglycaemic, antioxidant anti-diabetic, and analgesic properties.

Although the selected plants have been well studied and have a long history in traditional medicine, there are not many studies that investigate the nutritional quality for the possibility of inclusion in poultry nutrition.

The aim of the current study was to evaluate the bioactive compounds and antioxidant activity of poplar, turmeric and ginger extracts and to assess the potential use of selected phytogenic feed additives in animal nutrition.

MATERIALS AND METHODS

1.1. Preparation of Selected plants Extracts
Dried poplar buds (Populus nigra L.), rhizome of turmeric (Curcuma longa) and ginger (Zingiber officinale) were obtained from a local market and labelled. To analyze total phenolic content and total antioxidant capacity, the procedure for obtaining plant extracts used in this study was according to Unlea et al., (2018) with slight modifications. An amount of 0.5 g from each plant was extracted for 24 h in 20 mL of 80% methanol: distilled water solution (v:v). Following centrifugation, the extracts were stored at 4 °C for analyses. The extraction of lipophilic compounds (β-carotene, vitamin E) was performed according to Varzaru et al., (2015).

1.2. Proximate composition
The proximate composition (crude protein, crude fiber, ether extractives and crude ash) of plant samples was determined according to the chemical methods from Commission of the European Communities (2009). Crude protein, ether extractives, crude fibre and ash were determined according to ISO 5983-2/2009, SR ISO 6492/2001, ISO 6865/2002, ISO 2171/2010, respectively.

1.3. Phytochemical quantification
1.3.1. Estimation of total phenolic content
The total phenolic content of plant extracts was determined using the Folin–Ciocalteu method (Saracila et al., 2020). A calibration curve (r² = 0.9993) using a V-530 Jasco (Japan Servo Co. Ltd., Japan) spectrophotometer was prepared with a standard solution of gallic acid, and the final values were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry plant material (mg GAE/g dry plant).

1.3.2. Estimation of β-carotene content
Beta carotene assay was performed according to Varzaru et al., (2015) using a HPLC - Perkin-Elmer Series 200 Inc. high performance liquid chromatograph equipped with a Nucleodur C18 column at wavelength 450 nm.

1.3.3. Estimation of vitamin E content
Vitamin E was quantified according to the method described by Varzaru et al., (2020) using high performance liquid chromatography and a PDA-UV detector (HPLC Finningan Surveyor Plus, Thermo-Electron Corporation, Waltham, MA) at wavelength 292 nm.

1.3.4. Analysis of trace elements content
Trace elements (Cu, Fe, Zn, Mn) concentrations in selected plants were determined using flame atomic absorption spectrometry after wet digestion (H2NO3: H2O2) according to the method described by Untea et al., (2012). Trace elements concentrations were determined with a Thermo Electron – SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK) equipment and were expressed as mg/kg sample.

1.4. Antioxidant activity of plant extracts
The total antioxidant capacity of the selected plants was determined by the phosphomolybdenum method according to Prieto et al. (1999) with slight modification (Untea et al., 2018) using a V-530 Jasco (Japan Servo Co. Ltd., Japan) spectrophotometer. Calibration curves were prepared with standard solutions of ascorbic acid and vitamin E. The results were expressed as mmol equivalent vitamin C/kg and mmol equivalent vitamin E/kg.

1.5. Analysis of Data
All analyses were performed in triplicate and data is presented as mean ± standard error of the mean (SEM) values. To evaluate the differences between selected plant samples regarding each parameter analysed basic descriptive statistical analysis it was performed using one-way analysis of variance (one-way ANOVA) and Tukey’s HSD comparison test (95% significance level; p < 0.05) using XLSTAT (Addinsoft, Paris, France). Principal component analysis (PCA) was performed using XLSTAT software (Addinsoft, Paris, France) to
correlate variables to which nutritional parameters differed between selected plants.

**RESULTS AND DISCUSSION**

The proximate composition of plants is the first step in the chemical characterisation of a sample and allows to determine whether one ingredient is superior to another in terms of specific nutrients.

**Table 1.** Proximate composition of selected plants

<table>
<thead>
<tr>
<th>Specification, %</th>
<th>Poplar buds</th>
<th>Turmeric</th>
<th>Ginger</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>9.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ether extractives</td>
<td>3.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.006</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>27.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Crude Ash</td>
<td>2.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.017</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means in the same column without a common superscript differ (p<0.05).

The proximate analysis of selected plants (Table 1) showed that poplar buds had a higher content of crude protein, fat and fiber than turmeric and ginger. The content of crude protein, fat and fiber were as follows: poplar buds>turmeric>ginger. Turmeric showed a higher content of ash compared to the other plants. High ash content of turmeric highlights a higher content of trace minerals. Considering nutritional profiling of turmeric, our results are higher than those reported by other authors (Imoru et al., 2018; Mughal et al., 2019), except of crude protein which was lower. These results showed the valuable chemical composition of the selected plants and their suitability for use as ingredients in poultry diet. A mention can be made in the case of poplar buds that contain a large amount of crude fiber, so that in diets for birds they must be included in small percentages.

**Table 2.** Bioactive compounds of selected phytogenic feed additives

<table>
<thead>
<tr>
<th>Bioactive nutrient</th>
<th>Poplar buds</th>
<th>Turmeric</th>
<th>Ginger</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content, mg/g GAE</td>
<td>87.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.659</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β-carotene µg/g</td>
<td>2.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
<td>0.008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin E, µg/g</td>
<td>210.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.636</td>
<td>&lt;0.0001</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Mineral profile (mg/kg)</th>
<th>Copper</th>
<th>Iron</th>
<th>Manganese</th>
<th>Zinc</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>13.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>508.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>251.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>428.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.004</td>
<td>0.008</td>
<td>0.002</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means in the same column without a common superscript differ (p<0.05). GAE - gallic acid equivalents.
The bioactive compounds found in plants are responsible for their natural antioxidant activity, and the majority of these active compounds are polyphenols, vitamins, xanthophyll, etc. (Gazwi et al., 2022). Table 2 shows that poplar buds, turmeric and ginger extracts have different phytochemical compounds.

The extract of poplar buds had a significantly higher (p < 0.0001, Table 2) content of total polyphenols (87.20 mg of gallic acid/g) and vitamin E (210.53 µg/g) than in the extract of turmeric and ginger. The content of polyphenols in selected plants registered the following trend: poplar buds>turmeric> ginger. Previous evidence has showed similar content of total phenols in aqueous extract of balsam poplar buds (Stanciauskaite et al., 2021), higher content in ethanolic turmeric extract (Singh et al., 2022) and in ginger (60.34 mg GAE /g) extracted in petroleum ether and chloroform: methanol (Ali et al., 2018). Dudonné et al. (2011) identified phenolic acids and flavonoid aglycons as main phenolic compounds in poplar buds, representing about 50% of the total antioxidant activity of the extract.

The results from Table 2 reveal a very high amount of vitamin E in poplar buds (210.53 µg/g) compared to that in turmeric (3.72 µg/g) or ginger (1.85 µg/g) extracts, while similar concentrations were found in turmeric and ginger extracts. The beta carotene content was significantly higher in poplar buds (2.99 µg/g) than in turmeric (1.67 µg/g). In ginger, the beta carotene content was under detection limit. The bioactive compounds contained are the key contributors of overall antioxidant activity.

The presence of trace elements in feed components is essential for the normal physiological function of animals. Often these are not provided by the conventional raw materials from the animal feed in the necessary quantity, so that for many elements it is necessary to supplement from the synthetic source (in premix). Table 2 shows the concentration of minerals in selected plants. A large variability can be observed between the three analysed plants in terms of trace element content. For instance, turmeric showed the highest content in iron and copper; ginger showed the highest content in manganese and poplar buds had the highest content in zinc. The amount of iron in turmeric was 2 times higher than in ginger and approximately 10 times higher than in poplar buds. Manganese level in ginger was approximately 5 times higher compared to turmeric and 28 times higher compared to poplar buds. The most abundant element in the plant samples was iron and manganese. Iron and manganese were identified as possible nutritional components involved in many physiological processes (Abbaspour et al., 2014). Iron is vital to the proper function of haemoglobin, and a key element for growth and development (Abbaspour et al., 2014). Manganese is a coenzyme involved in carbohydrates, proteins, and cholesterol metabolism (Li et al., 2018).
Figure 1. Total antioxidant capacity of selected plant extracts expressed as mM equiv. vit C (figure A) and mM equiv. vit. E (figure B)

Figure 1 shows the antioxidant activity of poplar buds, turmeric and ginger extracts. In the present study, the antioxidant capacity of selected plant extracts was determined by their ability to reduce Mo (VI) to Mo (V) and to quantify the concentration of the phosphate/Mo(V) complex formed. It expresses the antioxidant capacity of nutrients such as phenolics, ascorbic acid, tocopherols and carotenoids from selected plants. To highlight the above-mentioned effect, we used two antioxidants (vitamin C and vitamin E) as standard solutions for calibration curves. It can be seen that poplar buds express a higher antioxidant capacity than turmeric and ginger. Moreover, poplar buds possessed the highest antioxidant capacity both on vitamin C and E calibration curve, evidencing the rich content in such antioxidants. Other studies showed the effective antioxidant activity of poplar buds (Stanciu et al., 2010; Dudonné et al., 2011) the major contributors being phenolic compounds including caffeic and p-coumaric acids. Merghache et al., (2016) showed that the best antioxidant effect was expressed at the concentration of 1 mg/mL similarly to the antioxidant activity of ascorbic acid. Literature findings revealed that the antioxidant effect of turmeric is attributable to curcumin, its main polyphenolic constituent (Gül and Bakht, 2015). Regarding ginger antioxidant activity, many scientists attributed that effect to phenolic compounds, gingerols, shogaols, minerals, vitamins (Oboh et al., 2012; Mustafa and Chin, 2023). The methanolic extract of ginger had a more pronounced antioxidant activity expressed as mM equiv. vit C than that of turmeric and the
reverse if it was expressed as mM equiv. Vit E (Figure 1). That observation demonstrated that hydrophilic antioxidant compounds express more pronounced total antioxidant capacity in ginger, while those lipophilic antioxidant compounds express better antioxidant capacity in turmeric. The antioxidant mechanism of plant extracts is different, they possibly act as effective antioxidants by inhibiting/ quenching free radical reactions, inhibiting prooxidative enzymes or chelating metals that convert iron and copper (metal prooxidants) into stable products (Sotler et al., 2019; Barreca et al., 2020).

![Biplot correlation from principal component analysis (PCA) of plant samples](image)

**Figure 2.** Biplot correlation from principal component analysis (PCA) of plant samples

Abbreviations: TAC, total antioxidant capacity; CP, crude protein; EE, ether extractives; CF, crude fiber.

The results of Figure 2 showed that 99.67% of the total variation is explained by the first two principal components (PC1 covered 73.09% of variance and PC2 covered 26.58% variance). According to biplot correlation, the most important variables in PC1 are polyphenols, vitamin E, Zn, TAC, CF, beta carotene, CP, Fe, Mn, and Ash. For the second PC, Cu and EE were the most important variables. Figure 2 also showed correlations between variables. According to squared cosines and correlation biplot, the polyphenols were
strong positively correlated with TAC, beta carotene, vitamin E, Zn, CP, EE, CF and negatively correlated with Fe, Mn and Ash. A strong correlation was seen in TAC vit C against polyphenols, beta carotene, vitamin E, Zn and CF based on their close positions. Hence, this means that antioxidant activity of plant extracts was attributable to those bioactive compounds, an expecting result due to the redox properties which allow for free radical scavenging, quenching of singlet oxygen or decomposition of peroxides (Martemucci et al., 2022). Several other studies have also reported strong correlation between hydrophilic, lipophilic antioxidants, minerals and antioxidant capacity (Teow et al., 2007). A negative correlation was seen in TAC vit C against Fe and Ash, whose locations were almost in the opposite of one another. Fe and Ash were also located on the opposite side of vit E, EE and CF. This result is in line with many other studies which showed that iron is a redox-active metal and participate in Fenton and Haber-Weiss reaction (Sotler et al., 2019). The biplot also discriminate the variables among selected samples. For instance, the biplot of PCA shows that polyphenols, vit E, TAC vit C and CF are the most definitory variables for poplar buds, Ash and Fe for turmeric and Mn for ginger.

CONCLUSION

In conclusion, all selected plants exhibit high quantity of bioactive compounds and promising antioxidant potential. Of all selected plants, poplar buds had the best antioxidant source due to high polyphenols and vitamin E content. Turmeric extract has characterized by higher Fe content and ginger by important content of Mn. Based on the nutritional quality confirmed through this study, poplar buds, turmeric and ginger can be recommended as phytogenic feed additives for poultry nutrition.

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