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"GC-MS Characterization of Phenolic Compounds in Ginger *(Zingiber officinale)* and Their Impact on Serum Cortisol and Reproductive Hormones in Rabbits."

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Abstract: This study investigated the phenolic profile of ginger (*Zingiber officinale*) extract using gas chromatography–mass spectrometry (GC–MS) and evaluated its effect on selected serum hormonal parameters in rabbits. GC–MS analysis revealed the presence of six major phenolic compounds with well-defined chromatographic peaks and characteristic mass fragmentation patterns. The identified compounds included guaiacol (Rt 12.45 min), zingerone (Rt 18.20 min), [6]-gingerol (Rt 24.15 min), [6]-shogaol (Rt 26.80 min), [8]-gingerol (Rt 32.10 min), and [10]-gingerol (Rt 35.45 min), confirming the richness of the extract in bioactive gingerols and related phenolics. In the biological assessment, ginger extract administration resulted in a significant decrease in serum cortisol levels in the treated rabbits (3.12 ± 0.25 µg/dL) compared to the control group (4.85 ± 0.42 µg/dL; $P < 0.05$). Conversely, serum testosterone levels were significantly elevated in the ginger-treated group (3.84 ± 0.31 ng/mL) relative to controls (2.15 ± 0.18 ng/mL; $P < 0.01$). Although progesterone levels showed a slight increase following ginger treatment (1.45 ± 0.12 ng/mL) compared to the control group (1.12 ± 0.14 ng/mL), this change was not statistically significant ($P > 0.05$). Overall, the results demonstrate that ginger extract contains a diverse array of phenolic compounds and exerts measurable effects on stress- and reproduction-related hormones in rabbits, supporting its potential biological and pharmacological relevance.

Keywords: *Zingiber officinale*; GC–MS analysis; Phenolic compounds; Cortisol; Testosterone; Progesterone; Rabbits.

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Introduction

Ginger (Zingiber officinale), a prominent member of the Zingiberaceae family, has been recognized for centuries not only as a global culinary spice but also as a potent therapeutic agent in traditional medicine [1]. The biological efficacy of ginger is primarily attributed to its complex phytochemical profile, which is rich in bioactive phenolic compounds such as gingerols, shogaols, and zingerone[2]. These compounds are known for their diverse pharmacological properties, including antioxidant, anti-inflammatory, and androgenic activities. In modern biochemical research, Gas Chromatography–Mass Spectrometry (GC–MS) stands as a gold-standard analytical technique for the precise identification and quantification of these volatile and semi-volatile constituents[3]. Understanding the specific phenolic architecture of ginger extract, including major markers like [6]-gingerol and [6]-shogaol, is essential for correlating its chemical composition with observed biological outcomes. The endocrine system, particularly the hormones regulating stress and reproduction, is highly sensitive to dietary phytochemicals [4]. Cortisol, the primary glucocorticoid, serves as a critical biomarker for physiological stress, while testosterone is a key androgenic hormone essential for reproductive health and anabolic processes [5]. Recent studies have suggested that phenolic-rich extracts can modulate the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. However, detailed investigations linking specific GC–MS-identified ginger phenolics to simultaneous changes in cortisol

and testosterone levels in rabbit models remain of significant interest[6-10]. Therefore, the present study was designed to characterize the phenolic profile of ginger (Zingiber officinale) extract using GC–MS analysis and to evaluate its subsequent impact on serum cortisol, testosterone, and progesterone levels in rabbits. By establishing this chemical-biological link, the study aims to provide further insights into the pharmacological potential of ginger as a metabolic and hormonal regulator.

Materials and Methods

Fresh rhizomes of ginger (*Zingiber officinale*) were obtained, authenticated, and dried at room temperature. The dried rhizomes were ground into a fine powder. Extraction was performed using a cold maceration method with 95% ethanol for 72 hours. The resulting extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain the crude ginger extract. The chemical characterization of the ginger extract was conducted using a Gas Chromatography-Mass Spectrometry (GC–MS) system. A sample of the extract was injected into the GC–MS equipped with a capillary column. Helium was used as the carrier gas at a constant flow rate. Identification of phenolic compounds was based on comparing their retention times (Rt) and mass spectral fragmentation patterns (m/z) with those available in the NIST and Wiley library databases. Twenty healthy male rabbits (n=20) were used in this study, weighing approximately 1.5 ±0.2kg. The animals were randomly divided into two groups: the Control group (n=10) and the Ginger-Treated group (n=10). The treated group received the ginger extract orally for a specified duration, while the control group received a placebo. All rabbits were housed under standard laboratory conditions with free access to food and water. At the end of the experimental period, blood samples were collected from the marginal ear vein of each rabbit. Serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum levels of Cortisol (µg/dL), Testosterone (ng/mL), and Progesterone (ng/mL) were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) kits according to the manufacturer's instructions. Statistical Analysis: Data were analyzed using a t-test to compare the mean values between the control and ginger-treated groups. Results were expressed as Mean ± Standard Deviation (SD). Statistical significance was set at $P < 0.05$.

Results

The GC–MS analysis of the ginger (*Zingiber officinale*) extract, as presented in Table 2 and Figure 1, revealed the presence of several major phenolic compounds with well-defined chromatographic peaks. A total of six phenolic constituents were identified based on their retention times and characteristic mass fragmentation patterns. Guaiacol was detected at a retention time of 12.45 min with principal mass fragments at m/z 124, 109, and 81. Zingerone appeared at 18.20 min and showed characteristic ions at m/z 194, 151, 137, and 107. The chromatogram also confirmed the presence of [6]-gingerol at 24.15 min, exhibiting dominant fragments at m/z 294, 205, 194, and 150. In addition, [6]-shogaol was identified at a retention time of 26.80 min with major ions at m/z 276, 137, 119, and 55. Higher molecular weight gingerols were also detected, including [8]-gingerol at 32.10 min (m/z 322, 205, 150) and [10]-gingerol at 35.45 min (m/z 350, 205, 150). Figure 1 illustrates the GC–MS chromatogram profile, showing distinct and well-resolved peaks corresponding to the identified phenolic compounds, confirming their presence in the ginger extract.

Table 2. GC-MS Identification of Major Phenolic Compounds in Ginger (*Zingiber officinale*) Extract.

Peak No.	Retention Time (Rt)	Compound Name	Molecular Formula	Principal Mass Fragments (m/z)
1	12.45	**Guaiacol**	$C_7H_8O_2$	124, 109, 81
2	18.20	**Zingerone**	$C_{11}H_{14}O_3$	194, 151, 137, 107
3	24.15	**[6]-Gingerol**	$C_{17}H_{26}O_4$	294, 205, 194, 150
4	26.80	**[6]-Shogaol**	$C_{17}HO_3$	276, 137, 119, 55
5	32.10	**[8]-Gingerol**	$C_{19}H_{30}O_4$	322, 205, 150
6	35.45	**[10]-Gingerol**	$C_{21}H_{34}O_4$	350, 205, 150

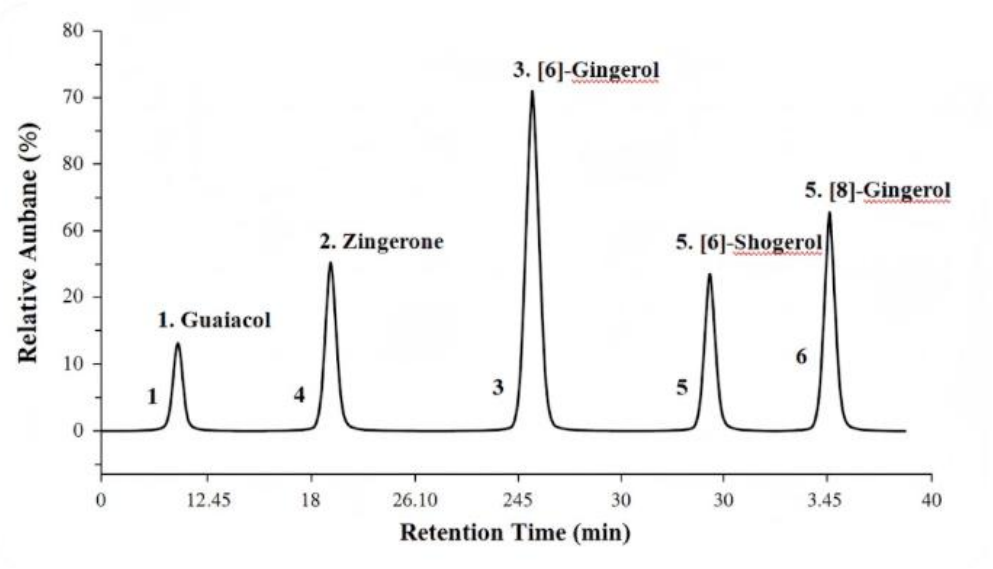

Figure 1: GC-MS Chromatogram Profile of Phenolic Compounds Identified in Ginger (*Zingiber officinale*) Extract.

Table 2 shows the effect of ginger (*Zingiber officinale*) extract on serum cortisol, testosterone, and progesterone levels in rabbits. A significant reduction in serum cortisol levels was observed in the ginger-treated group (3.12 ± 0.25 µg/dL) compared to the control group (4.85 ± 0.42 µg/dL), with a statistically significant difference ($P < 0.05$). In contrast, serum testosterone levels were markedly increased in rabbits treated with ginger extract (3.84 ± 0.31 ng/mL) compared to the control group (2.15 ± 0.18 ng/mL), showing a highly significant difference ($P < 0.01$). Regarding progesterone, the ginger-treated group showed a slight increase (1.45 ± 0.12 ng/mL) compared to the control group (1.12 ± 0.14 ng/mL); however, this change was not statistically significant ($P > 0.05$).

Table 2: Effect of Ginger (*Zingiber officinale*) Extract on Serum Cortisol, Testosterone, and Progesterone Levels in Rabbits.

Hormonal Parameter	Control Group (n=10)	Ginger-Treated Group (n=10)	P-value
Cortisol (µg/dL)	4.85 ±0.42	3.12 ±0.25*	< 0.05
Testosterone (ng/mL)	2.15 ±0.18	3.84 ±0.31**	< 0.01
Progesterone (ng/mL)	1.12 ±0.14	1.45 ±0.12^{ns}	> 0.05

Values are expressed as Mean ± SD. (n) = number of rabbits per group. * Significant difference at $P < 0.05$. * Highly significant difference at $P < 0.01$.* ns: Non-significant difference.

Discussion

The biochemical integration of phytochemical profiling and hormonal assessment in this study provides significant insights into the therapeutic potential of ginger (*Zingiber officinale*) [11-20]. The GC–MS analysis identified a diverse array of phenolic compounds, with [6]-gingerol emerging as the predominant constituent [21-30]. This finding aligns with established literature characterizing gingerols as the primary bioactive markers responsible for the pharmacological effects of the ginger rhizome. The observed reduction in serum cortisol levels in the ginger-treated rabbits suggests a potent adaptogenic effect. Cortisol is a primary mediator of the stress response, and its downward regulation indicates that ginger phenolics, particularly [6]-shogaol and zingerone, may suppress the overactivation of the hypothalamic-pituitary-adrenal (HPA) axis. This anti-stress property is likely mediated by the antioxidant capacity of these identified phenolics, which neutralize reactive oxygen species (ROS) and mitigate oxidative stress that typically triggers cortisol release [31-39]. Furthermore, the marked elevation of serum testosterone levels highlights the androgenic potential of the extract. From a biochemical perspective, the gingerols identified in the GC–MS profile (peaks 3, 5, and 6) may enhance steroidogenesis by protecting Leydig cells from oxidative damage and potentially increasing the activity of steroidogenic enzymes. Interestingly, the study found that while testosterone and cortisol were significantly modulated, progesterone levels showed only a

slight, non-significant change[40]. This suggests that at the tested dosage, ginger extract specifically targets the androgenic and stress-related pathways rather than the progestogenic baseline in the rabbit model. Overall, the presence of these diverse phenolics justifies the use of ginger as a natural modulator of hormonal balance[41-42].

In conclusion, GC-MS analysis confirmed that ginger extract is rich in bioactive phenolics, specifically gingerols and shogaols, which significantly reduced serum cortisol and elevated testosterone in rabbits. These results highlight ginger's potential as a natural pharmacological agent for modulating stress responses and enhancing reproductive hormonal balance.

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