Trop J Nat Prod Res, January 2026; 10(1): 6684 - 6690
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Bioactive Content, Antioxidant and Lactogenic Effects of Ethanol Extract of *Gymnema sylvestre* Leaves

I Gusti Karnasih[1], Lulut Sasmito[1], Ilmah Fakhriza[2], Rika Mahardika Sari[1], Rian Anggia Destiawan[3], Sholihatil Hidayati[4]

[1] *Health Polytechnic of Ministry of Health, Malang, East Java, Indonesia*
[2] *Master Program of Midwifery, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*
[3] *Medical Laboratory Technology Study Program, Faculty of Health Sciences, Universitas dr. Soebandi, Jl. Dr. Soebandi No.99, Jember, Indonesia.*
[4] *Pharmacy Study Program, Faculty of Health Sciences, Universitas dr. Soebandi, Jl. Dr. Soebandi No.99, Jember, Indonesia*

ARTICLE INFO

Article history:
Received 14 November 2025
Revised 02 January 2026
Accepted 08 January 2026
Published online 01 February 2026

ABSTRACT

Gymnema sylvestre leaves have been empirically used, especially by the local Madurese, to increase breast milk production or as a galactagogue. However, scientific evidence supporting this use remains limited. Hormones, including oxytocin and prolactin are fundamental mediators of mammary morphogenesis and lactogenesis. This study aims to investigate the lactogenic effect of *Gymnema sylvestre* leaves in postpartum rats. The *in vitro* antioxidant capacity of the extract was assessed using the the DPPH method, while the lactogenic effect, including oxytocin and prolactin levels, were evaluated in vivo in postpartum rats using the ELISA method. A total of 30 postpartum rats were divided into 5 groups: negative, positive, and treatment groups receiving GSLE doses of 25, 50, and 100 mg/kg BW, treated for 14 days. The results showed GSLE contains bioactive compounds, including flavonoids (89.2 ± 0.09 µg QE/mg), phenolic (53.9 ± 0.01 µg GAE/mg) and tannins (40.7 ± 0.01 µg GAE/mg). Antioxidant activity showed an IC_{50} value of 44.10 ± 3.12 µg/mL. The results of the lactogenic effect indicated that GSLE administration increased oxytocin and prolactin levels (p<0.05). Oxytocin levels increased significantly at the GSLE dose of 50 mg/kg BW on day 7. Prolactin levels were significantly increased by GSLE administration at doses of 25, 50, and 100 mg/kg BW compared to the negative control group. The antioxidant and lactogenic activities suggest that GSLE has the potential for further development as a galactogogue.

Keywords: Antioxidant, *Gymnema sylvestre*, Lactogenic, Oxytocin, Prolactin

Introduction

Breast milk is essential for optimal physical growth and neurological development of infants, providing nutrients and various non-nutritional bioactive factors.[1] Exclusive breastfeeding provides numerous benefits, including increased antibodies, protection against infections, and support for brain and immune system development.[2] The WHO has established a global health mandate recommending that infants be exclusively breastfed for a duration of six months, with a target of at least 50% coverage by 2025.[3] However, a small percentage of women are unable to achieve this due to a primary low milk supply. This condition remarks an intrinsic condition caused by factors such as insufficient glandular tissue, metabolic disease, hormonal imbalance, or insulin resistance.[4] This study highlights the high prevalence of galactagogue consumption among lactating women within the United States, particularly among first-time mothers, those considered to have insufficient milk, and mothers who rely on expressed breast milk.[5]

*Corresponding author. Email: hidayati.sho@uds.ac.id
Tel: +6282234358835

Citation: Karnasih IG, Sasmito L, Fakhriza I, Sari RM, Destiawan RA, Hidayati S. Bioactive Content, Antioxidant and Lactogenic Effects of Ethanol Extract of *Gymnema sylvestre* Leaves. Trop J Nat Prod Res. 2026; 10(1): 6684 – 6690 https://doi.org/10.26538/tjnpr/v10i1.35

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

To stimulate, sustain, and augment lactation, galactagogues can be either synthetic compounds or plant-derived molecules. Galactagogues treatment, administered orally or as medication, can help women with inadequate breast milk.[6] Herbal galactagogues can effectively raise levels of prolactin and oxytocin in the blood when taken one to two weeks postpartum. Their popularity is rising due to perceived safety and efficacy.[7] As an integral component of the reproductive continuum, lactation follows the stages of embryogenesis and mammogenesis, culminating in secretory differentiation and activation (lactogenesis I and II). These developmental milestones are regulated by synergistic interactions between steroid and peptide hormones, including estrogen, progesterone, prolactin, and oxytocin, among other metabolic regulators like insulin and hydrocortisone.[8] The physiological 'let-down' response is a neuroendocrine reflex elicited by infant suckling. Sensory impulses from the nipple areolar complex are transmitted via afferent nerves to the hypothalamus, facilitating the pulsatile secretion of prolactin for milk biosynthesis and oxytocin for milk propulsion.[9]

The local Madurese of Java Island have traditionally relied on *Gymnema sylvestre*, believed to stimulate breast milk production.[10] The *G. sylvestre*, a member of the Apocynaceae family, is formally known as *Gymnema sylvestre* (Retz.) R.Br. ex Sm. This woody, heavily branched plant can reach great heights and is native to the arid woodlands of Southern and Central India, as well as other parts of Asia.[11] The simple leaves, 2.5-6 cm long, are typically ovate or elliptical with distichous phyllotactic arrangement.[12] They are acute or briefly acuminate, with petioles 1-2 cm long, ciliate margins (particularly along the veins), and a smoother upper surface, a rounded base, and a densely velvety pubescent underside. The leaves feature a marginal vein and reticulate, transverse venation.[13]

G. sylvestre leaves contain flavonoids, primarily quercetin and kaempferol.[14] Quercetin reduces PI3K-Akt and NF-κβ signaling, thereby reducing mammary gland inflammation in mice.[15] In addition, quercetin administered to lactation-challenged mice (treated with bromocriptin) stimulated PRL production and increased the expression

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

of β-casein, stearoyl-CoA desaturase, fatty acid synthase, and β-lactalbumin. Furthermore, quercetin also promotes mammary epithelial cell proliferation and stimulates PRL receptor expression in vitro. A recent study showed that quercetin increased insulin resistance in mice, an inhibitor of milk production in humans.[16] These findings suggest that quercetin may influence mammary gland function through the regulation of hormonal signaling pathways.
Further study is required to delineate the pharmacological properties and biological underpinnings of galactagogue activity. This study aims to determine the bioactive components of *G. sylvestre*, *in vitro* antioxidant activity, and *in vivo* lactogenic effects in postpartum rats.

Materials and Methods

Materials
Bioassay Technology's Rat Oxytocin and Prolactin Receptor ELISA Kit, ethanol and methanol from Merck, DPPH from Sigma, gallate acid standard from Sigma, ethanol and methanol from Sigma, and quercetin standard from Sigma. The experiments utilized the following equipment: Memmer UN 55 oven, Shimadzu UV-1900i UV-VIS Spectrophotometer (Germany), and an Elisa Reader (EPOCH Bio Tek, USA).

Collection and Identification of Plant materials
G. sylvestre samples were collected in Bintoro Village, Jember City, East Java, Indonesia, at coordinates 8º13'9.705" S, 113º72'0.638" E on June 2024 (Figure 1 and 2). The plants were authenticated by Faculty of Applied Science and Technology, Universitas Ahmad Dahlan with voucher number 625/Lab.Bio/B/XII/2025.

Figure 1: *G. sylvestre* plant and leaf

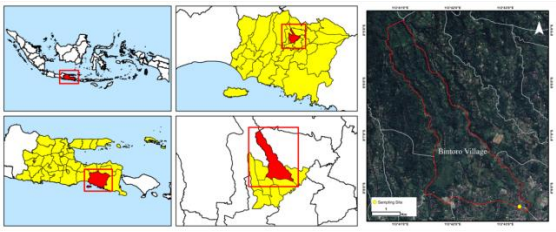

Figure 2: Study area location map with the sampling sites

Extraction of G. sylvestre
The leaves were washed were thoroughly cleansed under running tap water to remove surface debris, followed by a final rinse with distilled water to ensure purity. Subsequently, they were subjected to thermal dehydration in a laboratory oven maintained at 50°C until constant weight was achieved. Dried leaves 100 g was extracted with 1000 mL of of 96% ethanol. Extraction was performed using ultrasonication for 1 h at 50ºC with power 600 W and frequency 40 KHz, followed by two additional rounds of ultrasonication. After maceration, the collected solution was concentrated using a rotary evaporator set at 50ºC. The filtrate was further concentrated using a water bath until a constant-weight extract was obtained.[17] The resulting extract was stored at 25ºC temperature in a light-proof, tightly closed container.

Flavonoid, phenolic and tannin level evaluation of G. sylvestre leaves extract
The bioactive components of GLSE were assessed, with flavonoid content determined using a colorimetric technique. GSLE was solubilized in ethanol, followed by the addition of $NaNO_2$ and a 5-minute incubation. Then, 0.3 mL of 10% $AlCl_3$ was incorporated into the mixture and allowed to react for an additional 5 minutes. Subsequently, the reaction was terminated by adding 2 mL of 1 M NaOH and diluted with 10 mL of distilled water. Following a standardized incubation protocol, the optical density of the resulting chromophore was measured spectrophotometrically at a wavelength of 510 nm. To ensure accuracy, a five-point calibration curve was generated using quercetin standards ranging from 20, 40, 60, 80, to 100 µg/mL, following the same procedure. The flavonoid concentration was subsequently normalized and reported in QE units per milligram µg/mg of dry extract weight.[18]
To evaluate the phenolic concentration, GSLE (20 mg) was prepared in a methanolic solvent (60% methanol, 3% HCl). A 100 µL volume of this solution was combined with 2 mL of aqueous Na_2CO_3 and left to stand for three minutes. Afterward, 100 µL of Folin–Ciocalteu phenol reagent was added, and the mixture was incubated for 30 minutes. Absorbance was measured at 750 nm. A standard curve utilizing gallic acid 0.5, 1.0, 1.5, 2.0, and 2.5 mM, served as the reference for expressing phenolic content (µg GAE/mg).[19]
Tannin content was assessed by dissolving 100 mg of extract in 100 mL of distilled water. Following extraction, 5–10 drops of the solution were mixed with 1 mL of Folin–Ciocalteu reagent, agitated, and left to stand for 5 minutes, followed by 2 mL of 15% Na_2CO_3 solution was added, the mixture was shaken thoroughly, and left to stand for another 5 minutes. The reaction was stabilized for 5 min before being diluted with 10 mL of distilled water for spectrophotometric analysis at the peak wavelength. Measurements were performed three times. Tannin content was determined as gallic acid equivalent.[20]

In vitro antioxidant activity by the DPPH method
Antioxidant activity was evaluated using the DPPH free radical scavenging method. GSLE was diluted with methanol to concentrations of 2.5, 5, 10, 20, 40, and 80 µg/mL, with 3 replicates. A UV-Vis spectrophotometer reading of 517 nm was taken after 30 minutes of incubation after the samples were treated with 1 mL of a 0.2 mM solution of DPPH.[21] Quercetin was used as a positive control following the same procedure. Antioxidant activity was expressed as the percentage inhibition of radical formation, calculated from the absorbance values (Ab).
The DPPH inhibition of a sample is used to measure its antioxidant activity:

$$\% \text{ Inhibition} = \frac{\text{Ab Control} - \text{Ab Sample}}{\text{Ab Control}} x100\%$$

The IC_{50} was determined by interpolating the 50% inhibition percentage from a linear regression of the concentration-inhibition relationship. The antioxidant capacity was quantified as the IC_{50}, value, defined as the specific extract concentration necessary to inhibit 50% of the initial DPPH radical population. A lower IC_{50} value indicates higher radical scavenging potency.[22,23]

In vivo activity evaluation of oxytocin and prolactin serum by the ELISA method
Thirty pregnant Wistar rats (3–4 months old, body weight 250–350 g), were randomly assigned to five experimental groups (n=6). The negative control group consisted of postpartum rats receiving distilled water as a vehicle. The positive one was treated with 65 mg/kg BW of Domperidone. The three treatment groups were administered GSLE at daily oral doses of 25, 50, and 100 mg/kg BW, respectively. A single oral dose was administered daily for 14 days, starting on the first day postpartum. This study was conducted in accordance with the ethical guidelines approved by the Health Research Ethics Committee of the State Polytechnic of Health Malang no.814/KEPK-POLKESMA/2024. Blood samples were obtained from the animals through the sublingual vein on days 0, 7, and 14. Samples underwent centrifugation at 3000

rpm for a duration of 10 min. For oxytocin measurement using the ELISA technique, serum was transferred from the clot into clean tubes with a Pasteur pipette. The resulting serum was analyzed for prolactin and oxytocin levels using rat-specific ELISA kits sourced from Bioassay Technology Laboratory (Shanghai, China). All assays were conducted in accordance with the provided instructions to ensure analytical accuracy and reproducibility.
Absorbance values from the standards were used to generate a linear regression curve against concentration in Microsoft Excel, yielding the equation $y = ax + b$. Sample absorbance values were subsequently applied to the equation to determine oxytocin and prolactin concentrations. All tests were performed in triplicate.

Statistical analysis
Data on the bioactive content of GSLE, including flavonoids, phenolics, and tannins, are displayed as mean values. Antioxidant activity is expressed as mean values and reported as IC_{50}. Lactogenic activity was evaluated based on prolactin and oxytocin levels. Differences between groups were statistically analysed using SPSS 25.0 with one-way ANOVA followed by Tukey's tests ($p<0.05$).

Results and Discussion

The *G. sylvestre* plant is sourced from the Jember area**.** Figure 1 shows its leaves. The antioxidant capabilities of *G. sylvestre* leaves are due to compounds (flavonoids, cinnamic acid, folic acid, ascorbic acid, and others).[24]
G. sylvestre has been used as a vegetable and a medicinal plant for various ailments for centuries. Its leaves have been employed as a diuretic in herbal teas and as a remedy for constipation and intestinal ulcers in a decoction. Indigenous practitioners believe that nursing mothers can improve their milk production by consuming chopped and boiling leaves. Similarly, new mothers in Sri Lanka consider the leaves a food supplement to boost breast milk production.[25]
The lactogenic use of *G. sylvestre* is also supported by ethnomedicine practices in several regions of Indonesia. Empirically, the Madurese people in Jember City use a stew of *G. sylvestre* leaves as a traditional herb to increase milk production in postpartum mothers. In Bali, this plant is incorporated into postpartum herbal medicine to support and facilitate breastfeeding.[26]

Flavonoid, phenolic and tannin evaluation of G. sylvestre extract
Quercetin and gallic acid standards were used to assess flavonoid content and tannin and phenolic levels, respectively. Table 1 displays the results of the bioactive content assessment. Phenolic compounds exist in two forms: soluble and insoluble. Some phenolic compounds are unbound and readily available, whereas others are bound to molecules.[27] The availability and potential bioactivity of phenolic and flavonoid compounds differ between the bound insoluble fraction and the free soluble fraction. Increasing the amount of the free soluble form enhances absorption in the small intestine, potentially improving overall bioavailability.[15]

Table 1: Flavonoid, phenolic and tannin level results of GSLE

Bioactive compound		Equations	R^2	Average ± SE (n=3)
Flavonoid	(µg QE/mg)	y=0.0682x+0.0050	0.9990	89.2±0.09
Phenolic	(µg GAE/mg)	y=0.0108x+0.0017	0.9994	53.9±0.01
Tannin	(µg GAE/mg)	y=0.0581x+0.0122	0.9974	40.7±0.01

The current study showed that GSLE contains 89.2±0.09 µg QE/mg extract of flavonoids. Phonelic content was 53.9±0.01 µg GAE/mg extract, and tannin content was 40.7±0.01 µg GAE/mg extract (Table 1). Flavonoids, as polyphenolic compounds, are soluble in polar solvents and partially soluble in semipolar solvents.[28] Numerous studies have examined a wide range of bioactive effects of flavonoids, including anti-inflammatory, cardioprotective, and antibacterial activities.[29,30]
Phenolic compounds are known to act as potent antioxidants by breaking free radical chains. Their hydroxyl groups enable effective free radical scavenging, which underlies their antioxidant activity.[31]
Dietary intake of up to 1 g of polyphenolic compounds per day from fruits and vegetables may help reduce mutagenesis and carcinogenesis. Total phenols, present in both the root and entire plant of *G. sylvestre*, play a crucial role in oxidation regulation.[32]
Another study reported that *G. sylvestre* extract contains 22.94 ± 0.66 mg/mL of flavonoids.[33] This difference likely reflects differences in solvent and standard used, indicating that ethanol extracts of *G. sylvestre* contain higher amounts of quercetin-type flavonoids. Simple flavonoid structures (aglycones), such as quercetin, are more soluble in alcohol solvents.[34]

Evaluation of in vitro antioxidant activity of G. sylvestre extract
The *G. sylvestre* antioxidant activity was evaluated in vitro using DPPH inhibition. The findings indicated that the leaf extract has an antioxidant capacity of 44.10 ± 3.12 µg/mL, categorizes the extract as a "strong" (Table 2). Antioxidant capacity is expressed as IC_{50}, with classifications: very strong (<50 µg/mL), strong (50–100 µg/mL), moderate (100–150 µg/mL), and weak (150–200 µg/mL).[35]

Table 2: *In vitro* activity of the antioxidant activity of GSLE

Sample	Rep	IC_{50}	Average (µg/mL)±SE
GSLE	1	50.20	44.10±3.12[a]
	2	39.94	
	3	42.15	
Quercetin	1	2.10	2.06±0.02[b]
	2	2.03	
	3	2.03	

The chemical components found in GSLE are responsible for its antioxidant action. Flavonoids, a class of specialized secondary metabolites composed of diverse polyphenolic compounds, contribute to the color, aroma, and flavor of plants, fruits, vegetables, grains, and seeds.[36] Flavonoids influence OS in biological systems through multiple mechanisms, exhibiting potent antioxidant effects.
Both direct and indirect ways of controlling oxidation underlie flavonoids' protective effects in the body. Although the primary mechanisms remain unclear, recent studies suggest that flavonoids modulate concentrations of ROS and RNS in biological systems.[37]
While the antioxidant capacity of most flavonoids is fundamentally derived from their stable backbone architecture, their radical scavenging efficacy is significantly modulated by specific substituents and metabolic biotransformation. Beyond direct scavenging, flavonoids attenuate the production of inducible NO. Although NO is an essential mediator of vasodilation, neurotransmission, and immunoregulation, its unregulated interaction with superoxide radicals generates the highly cytotoxic oxidant peroxynitrite. In this context, flavonoids serve as critical antioxidants by neutralizing ROS, thereby inhibiting the synthesis of peroxynitrite and maintaining NO homeostasis.[39]
The antioxidant activity of *G. sylvestre* showed IC_{50} value of 74.8 µg/ml using the ABTS method and 83.8 µg/ml using the DPPH assay.[40]
Another study states that *G. sylvestre* has an antioxidant capacity equivalent to ascorbic acid.[41]

Evaluation of the in vivo lactogenic effect of G. sylvestre extract on oxytocin level
A woman's body and mind undergo remarkable transformations during pregnancy. Hormone levels, including estrogen and progesterone, are known to fluctuate during pregnancy.[42] Oxytocin is a peptide hormone synthesized in the hypothalamic SON and PVN that functions as a critical regulator of the let-down reflex. Its secretion is essential for the mechanical and physiological aspects of nursing, serving as a primary

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

link between neural stimuli and the endocrine response in the lactating breast. Oxytocin levels increase with gestational age.[43] Research in cows shows plasma oxytocin levels reach a comparable high on delivery day but remain relatively steady and low in the bloodstream during the last stages of gestation.[44] This study shows that oxytocin levels increased significantly following 50 mg/kg BW GSLE on day 7 of study (Table 3 and Figure 3).

Table 3: Oxytocin standard linear regression

Concentration (ng/mL)	Absorbance	Equations	R2	LoD	LoQ
32	0.804	y = 0.0211x + 0.0823	0.9998	0.5977	1.9922
16	0.418				
8	0.252				
4	0.172				
2	0.120				

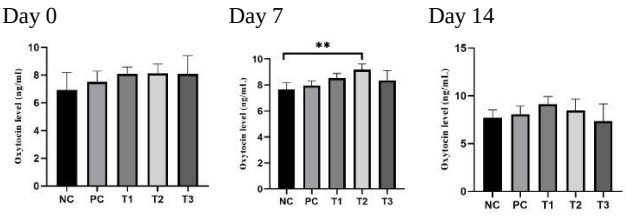

Figure 3: Oxytocin levels in postpartum mice after GSLE administration of doses of 25, 50 and 100 mg/kg BW. NC (Negative control), PC (positive control), T1 (postpartum rats with GSLE treatment dose 25 mg/kg BW), T2 (postpartum rats with GSLE treatment dose 50 mg/kg BW), T3 (postpartum rats with GSLE treatment dose 100 mg/kg BW). Data were statistically analysed with a significance value at $p < 0.05$.

During lactation, systemic oxytocin release is critical for the milk-ejection reflex, as it induces the contraction of myoepithelial cells encompassing the mammary alveoli. Simultaneously, this hormone induces the relaxation of the lactiferous duct sphincters, thereby optimizing the transport of milk through the mammary architecture. This coordinated neuroendocrine response is fundamental to successful breastfeeding outcomes.[45] During lactation, oxytocin triggers milk production for children.[46] while prolactin promotes milk synthesis in the mammary glands.[47] The neuropeptide oxytocin is synthesized within the paraventricular and supraoptic nuclei of the hypothalamus. Following synthesis, it is transported to the posterior pituitary gland, where it is subsequently secreted into the systemic circulation. This hypothalamic-hypophyseal pathway is critical for the regulation of various physiological and behavioral processes in response to stress, uterine dilation, various forms of social interaction, sexual stimulation, and labor.[48]

The results of a study on the administration of natural plant *Dracaena arborea* extracts showed inhibition of dopaminergic and oxytocinergic receptors.[49] Other research suggests that herbal administration can inhibit uterine contractions by affecting oxytocin levels in the body, including the spinal cord, thereby increasing breast milk production.[50] This oxytocin release barrier may be related to dopamine blockade that can alter the conformation or availability of oxytocin and interfere with downstream oxytocin signaling, resulting in oxytocin being produced but not effectively released.[51] A study comparing plasma oxytocin levels in patients receiving antipsychotics with dopamine receptor blockade showed lower oxytocin levels accompanied by higher prolactin levels.[52]

Evaluation of the in vivo lactogenic effect of G. sylvestre extract on prolactin level

Prolactin level (PRL) is a 23 kDa globular protein hormone characterized by a single-chain polypeptide sequence of 199 amino acids. The structural integrity of the human PRL molecule is maintained by 3 intramolecular disulfide bridges involving six cysteine residues at positions Cys4–Cys11, Cys58–Cys174, and Cys191–Cys199. Functionally, this polypeptide hormone is a primary mediator of mammary gland development and the initiation of lactation.

After postpartum, rat prolactin levels in all groups did not differ significantly. On the seventh day after treatment, prolactin levels differed significantly ($p<0.05$) in the GSLE treated group at doses of 50 mg/kg BW and 100 mg/kg BW compared with both the negative and positive control groups. On day 14, the groups receiving a positive control, 25 mg/kg BW of GSLE, or 50 mg/kg BW of therapy differed significantly ($p<0.05$) between negative controls. This demonstrates that GSLE at a dose of 50 mg/kg BW increases prolactin levels, making it an efficient lactogenic stimulator (Table 4 and Figure 4).

Lactation, breast growth, and other homeostasis-related functions are regulated by the polypeptide hormone prolactin. Growth hormone and placental lactogen share a structural similarity. They are members of the helical bundle protein-containing "prolactin/growth hormone/placental lactogen" family.[53] Prolactin is produced through post-translational modifications and proteolytic cleavage of the prohormone prolactin's signal peptide, resulting in a hormone composed of 199 amino acids.[54] Hormone synthesis in the anterior pituitary is regulated by the hypothalamus through dopamine. Furthermore, the uterus, immune system, central nervous system, and mammary glands can also contribute to prolactin production. These tissues initiate prolactin production in response to nipple stimulation, light, scent, and stress. Estrogen, thyrotropin-releasing hormone, and dopamine antagonists are among other variables that increase prolactin production.[55]

Table 4: Prolactin standard linear regression

Concentration (ng/mL)	Absorbance	Equations	R2	LoD	LoQ
2.40	0.910	y = 0.3505x + 0.055	0.9962	0.1966	0.6554
1.20	0.441				
0.60	0.277				
0.30	0.162				
0.15	0.115				

While involved in diverse physiological processes, prolactin's primary functions center on mammary gland development and subsequent milk production. The hormone facilitates the expansion of the mammary alveolar system and triggers the synthesis of essential milk components, including lipids, proteins (casein), and carbohydrates (lactose), within the alveolar epithelium. This lactogenic signaling is modulated by the steroid environment; specifically, high concentrations of progesterone serve to down regulate prolactin receptor expression, thereby inhibiting premature milk secretion.[57]

Meanwhile, the anterior pituitary gland is the main organ responsible for prolactin production. While prolactin is most recognized for its involvement in lactation and breast growth, it exerts a wide range of physiological impacts throughout the body. Dopamine inhibits prolactin synthesis and release by acting as an inhibitor of this hormone. This inhibitory action is an essential regulatory mechanism for maintaining prolactin levels in the body. Figure 3 shows that on days 7 and 14, the dopamine antagonist used as the positive control increased prolactin levels.[58]

GSLE treatment also showed a prolactin-binding effect on days 7 and 14, similar to the positive control and GSLE at 25 mg/kg BW showed a significant increase on day 14. Administration of GSLE at a dose of 50 increased prolactin levels on days 7 and 14. Administration of GSLE at 100 mg/kg BW increased prolactin levels on day 7. However, prolactin levels decreased on day 14 (Figure 3). Flavonoids exhibit a biphasic

effect on prolactin levels, showing *antiprolactinemic* effect at high doses and hyperprolactinemic effects at low doses. At low doses, flavonoids can act as estrogen mimetics by binding to estrogen receptors and stimulating prolactin release. Estrogen is known to inhibit dopaminergic activity and directly stimulate prolactin release in the anterior pituitary.[59] In contrast, flavonoids such as quercetin, luteolin, and apigenin at high doses tend to increase dopaminergic activity as indirect dopamine agonists and increase dopamine D2 receptor expression. Prolactin secretion is tonically suppressed by dopaminergic signaling originating from the tuberoinfundibular pathway. This neuroendocrine mechanism is a critical determinant of systemic prolactin homeostasis.[60] Therefore, dopamine blockade can increase prolactin secretion.

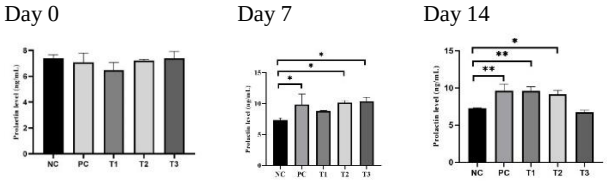

Figure 4: Prolactin levels in postpartum mice after GSLE administration of doses of 25, 50 and 100 mg/kg BW. NC (Negative control), PC (positive control), T1 (postpartum rats with GSLE treatment dose 25 mg/kg BW), T2 (postpartum rats with GSLE treatment dose 50 mg/kg BW), T3 (postpartum rats with GSLE treatment dose 100 mg/kg BW). Data were statistically analysed with a significance value at $p < 0.05$.

Research on lactogenic effects on fenugreek consumption shows that it affects the initial phases of lactogenesis and systemic prolactin concentrations but does not affect prolactin changes in later stages.[61] Mothers of premature neonates required to NICU, consumption of galactogog herbal tea improves lactation and prevents breast milk deficiency without side effects.[62] The findings from this study indicate that GSLE exhibits strong antioxidant and affects prolactin secretion.

The results of the examination indicate that the GSLE-induced increases in prolactin (PRL) levels can be explained by two intersecting molecular pathways. First, dopaminergic modulation occurs through reduction of dopamine inhibitor pressure on lactotrophs. Second, phytoestrogenic effects involve estrogen mimicry that reduces the activity of tubero-infundibular dopaminergic neurons, thereby reducing PRL inhibition. These pathways do not directly affect prolactin; rather, flavonoids, the main content of GSLE, namely quercetin, kaempferol has the potential to affect both.[63]

Through the dopaminergic pathway (D2 receptor/dopaminergic tone towards PRL), PRL secretion is physiologically tonically inhibited by dopamine released from tubero-infundibular neurons. Dopamine acts through D2 receptors on anterior pituitary lactotrophic cells, thereby suppressing PRL synthesis and release.[64] Therefore, any compound that reduces dopaminergic signalling or decreases dopamine availability in the pituitary cleft can remove this barrier and raise PRL levels.

Research also shows that flavonoids can interact directly with dopamine receptor proteins or modulate components of the dopaminergic system.[63] *In silico* and *in vivo* studies on quercetin show a tendency to D2 receptor binding and phenotypic changes consistent with dopaminergic modulation.[65] Molecular docking research further report that quercetin has affinity for D2 binding sites similar or adjacent to classical antagonists.[65] These findings support the hypothesis that quercetin and its derivatives in GSLE may decrease dopamine-mediated inhibition of PRL secretion.

Flavonoids are phytoestrogens or exhibit antagonistic agonist activity on estrogen receptors (ERα/ERβ) and related signaling pathways, with effects that are often dose-dependent and biphasic. Systematic studies of estrogenic flavonoids describe that flavonoid classes such as quercetin and kaempferol can mimic estrogen in some tissues and modulate pathways interacting with dopaminergic regulation.[66] Because estrogen is known to suppress tuberoinfundibular dopaminergic (TIDA) tone, thereby facilitating PRL release, estrogen-like flavonoid effect is a plausible pathway for increased PRL levels following GSLE treatment.

At low doses, flavonoids act as estrogen mimics that increase ER activity and decrease dopaminergic tone, resulting in increased PRL levels. In contrast, at high doses, some flavonoids increase dopaminergic activity or express anti-estrogenic effects. These results align with the results of this study, in which a moderate dose of 50 mg/kg BW increased PRL levels, while the highest dose (100 mg/kg BW) did not produce a sustained increase. This effect is likely due to a shift from estrogenic effects to anti-estrogenic dopaminergic effects at higher doses. Previous studies on quercetin support complex interactions among estrogenic activity, dopamine receptor modulation, and downstream signaling (PI3K/AKT, NF-κβ) all of which can influence PRL secretion.[66]

It can be deduced that the mechanism of GSLE is associated with its high flavonoid content, particularly quercetin and kaempferol derivatives. Quercetin can bind to or modulate D2 receptors function, as demonstrated by *in silico* docking and *in vivo* animal studies showing reduced dopaminergic responses. These results explain how D2 inhibition leads to increased PRL levels. Simultaneously, flavonoids can stimulate the ER or related pathways, suppress tuberoinfundibular dopamine neuron activity, reduce dopamine release to the pituitary gland and thereby release inhibitions of lactotroph cells, resulting in increased PRL secretion..

Conclusion

G. sylvestre leaves are a potential plant as a traditional medicinal plant containing bioactive compounds, including flavonoids (89.2±0.09 µg QE/mg), phenolic (53.9±0.01 µg GAE/mg) and tannins (40.7±0.01 µg GAE/mg). The antioxidant activity of this plant shows strong antioxidant capacity, and lactogenic activities of GSLE indicate its potential to enhance breast milk production. The findings of this study indicate that *G. sylvestre* leaves extract possesses strong antioxidant activity and demonstrates lactogenic effect. These effects may be associated with its rich content of flavonoids, phenolics, and tannins. Further studies are warranted to identify the active compounds and clarify the underlying mechanisms, particularly in relation to hormonal regulation involved in lactation. Additional *in vivo* studies and clinical validation are required to confirm its efficacy, safety, and potential application as a natural lactogenic agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was funded by the Indonesian Ministry of Health under Superior Research for Higher Education 2024 with number KU. 03.04/F.XXI.16.4/1//2024.

References

1. Neville MC, Demerath EW, Hahn-Holbrook J, Hovey RC, Martin-Carli J, McGuire MA, Newton ER, Rasmussen KM, Rudolph MC, Raiten DJ. Parental factors that impact the ecology of human mammary development, milk secretion, and milk composition-a report from "Breastmilk Ecology: Genesis of Infant Nutrition (BEGIN)" Working Group 1. Am J Clin Nutr. 2023;117:S11–27.
2. Alotiby AA. The role of breastfeeding as a protective factor against the development of the immune-mediated diseases: A systematic review. Front Pediatr. 2023;11:1086999.
3. World Health Organization. Indicators for the global monitoring framework on maternal, infant and young child nutrition. Geneva: WHO. 2014.

4. Whelan C, O'Brien D, Hyde A. Breastfeeding with primary low milk supply: a phenomenological exploration of mothers lived experiences of postnatal breastfeeding support. Int Breastfeed J. 2025;20(1).
5. Ryan RA, Hepworth AD, Lyndon A, Bihuniak JD. Use of galactagogues to increase milk production among breastfeeding mothers in the United States: A descriptive study. J Acad Nutr Diet. 2023;123(9):1329–1339.
6. Singh SP, Mukadam SS, Bisht A. An extensive investigation into the bioactive component of breast milk, lactation, and clinical application of Galactagogues: A review. J Phytopharmacol. 2024;13(2):114–121.
7. Budiono B, Pertami SB, Arifah SN, Atho'illah MF. Lactogenic effect of *Polyscias scutellaria* extract to maintain postpartum prolactin and oxytocin in lactating rats. J Ayurveda Integr Med. 2023;14(2):100580.
8. Abruzzese GA, Arbocco FCV, Ferrer MJ, Silva AF, Motta AB. Role of hormones during gestation and early development: pathways involved in developmental programming. In Advances in Maternal-Fetal Biomedicine: Cellular and Molecular Mechanisms of Pregnancy Pathologies. Springer; 2023. p. 31–70.
9. Rawal P, Ramesh C, Sowmya BA, Ranjitha CJ, Maria LA. Study on of Ethanol and Aqueous Extracts of *Embelia ribes* for Anthelmintic Properties. Asian J Rese Med Pharm Sci. 2023;12(4):1-6.
10. Vinydea YI, Atik S, Karnasih IGA, Fakhriza I. Breast Emptying with Breast Pump Increases Working Mother's Breast Milk Production. Indonesian Health Issue. 2025;4(1):58–68.
11. Abdulrazaq B, Ragosta S, Vijendrakumar BK, Ramakrishnan S. Comprehensive Review on Origin, Distribution, Micropropagation and Agronomical Practices of *Gymnema sylvestre* R. Br. Int J Environ Clim Chang. 2023;13(8):441–454.
12. Kraus Jr V, Čižmárová B, Birková A. When Your Body Tells You to Not Breastfeed-The Connivance of Oxytocin, Prolactin, and Dopamine. Inter J Mol Sci. 2025;26(12):5909.
13. Rajput KS, Rami M, Rahangdale SS, Rahangdale SR, Raole VM. Stem anatomy and ontogeny of phloem wedges in some species of *Gymnema* (Apocynaceae). Plant Biosyst. 2024;158(3):365–379.
14. Kalariya KA, Mevada RR, Das M. Characterization of phenylalanine ammonia lyase and revealing flavonoid biosynthesis in *Gymnema sylvestre* R. Br through transcriptomic approach. J Genet Eng. Biotechnol. 2024;22(1):100344.
15. Haryoto H, Trinanda E, Santoso B, Nagara MD, Liwanda N, Nurcholis W. Phytochemical Composition and Comparative Antioxidant Activity of Leaf and Stem Bark Extracts of *Glochidion arborecens* Using DPPH and ABTS Assays. Chem Eng Trans. 2025;115:19-24.
16. Mansour FR, Abdallah IA, Bedair A, Hamed M. Analytical methods for the determination of quercetin and quercetin glycosides in pharmaceuticals and biological samples. Crit Rev Anal Chem. 2025;55(1):187–212.
17. Hidayati S, Agustin AT, Sari Enik, Sari SM, Destiawan RA, Silvana WA. Phytochemical profiling and antidiabetic evaluation of *Peperomia pellucida* as a potential alpha glucosidase inhibitor. Biodiversitas. 2023;24(11).
18. Rofiqi AA, Astuti RI, Batubara I. The Potency of *Adenostemma platyphyllum* and A. madurense as Antioxidant and Anti-aging Agents. Trop J Nat Prod Res. 2025;9(1), 24–30.
19. Riskianto, Andriyani, Munthe SW, Novia J, Dillak SA, Jayanti T, Laksana SM, Langi AJ, Pattiradjawane Z, Sihotang F. Antihyperglycemic Activity, Total Phenolic, and Total Flavonoid Contents of 96% Ethanol Extract of Pisang Batu (*Musa balbisiana* Colla) Leaves. Trop J Nat Prod Res. 2025;9(1), 348–354.
20. Pascotto K, Hallé H, Watrelot A, Roland A, Meudec E, Carrillo S, Robillard B, Sommerer N, Poncet-Legrand C, Cheynier V. Characterization of enological oak tannin extracts by a multi-analytical approach. OENO One, 2025;59(2).
21. Muddapur UM, Manjunath S, Alqahtani YS, Shaikh IA, Khan AA, Mannasaheb BA, Yaraguppi D, More SS. Exploring bioactive phytochemicals in *Gymnema sylvestre*: biomedical uses and computational investigations. Separations. 2024;11(2):50.
22. Jain S, Vaidya A. Comprehensive review on pharmacological effects and mechanism of actions of taxifolin: A bioactive flavonoid. Pharmacol Res Mod Chin Med. 2023;7:100240.
23. Azeem M, Niazi S, Fayyaz F, Zahoor A, Zehra T, Fatima M, Abid HM. An overview of anticancer, anti-inflammatory, antioxidant, antimicrobial, cardioprotective, and neuroprotective effects of rutin. Curr Pharm Res. 2024;2(2):119-155.
24. Palaiogiannis D, Chatzimitakos T, Athanasiadis V, Bozinou E, Makris DP, Lalas SI. Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. Oxygen. 2023;3(3):274-286.
25. Weerasinghe D, Brough L, Everett DW, Rashidinejad A. *Gymnema lactiferum*: A Review of Its Traditional Applications, Phytochemical Constituents, and Biological Properties. Food Sci Nutr. 2024;12(11):8742–8754.
26. Karnasih IGA. Effectiveness of Leininger's Transcultural-based Gather Counseling Model on Exclusive Breastfeeding. Health Notions. 2021;5(5):156–162.
27. Shahidi F, Hossain A. Importance of insoluble-bound phenolics to the antioxidant potential is dictated by source material. Antioxidants. 2023;12(1):203.
28. Sharma D, Yusuf M, Asif M. *Gymnema Sylvestre*: Phytochemistry, Pharmacology and Economical Perspectives. J Adv Pharmacogn. 2024;4(2):78-90.
29. Hussen EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. BMC complement med ther. 2023;23(1):146.
30. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. Processes. 2023;11(8):2248.
31. Hartanti D, Romdhoni MF, Hamad A. Crude Drug Standardization and Antioxidant Properties of an Indonesian Antidiabetic Polyherbal Formulation and Plant Constituents. Trop J Nat Prod Res. 2025;9(5):2074–2080.
32. Liu Y, Wang X, Podio NS, Wang X, Xu S, Jiang S, Wei X, Han Y, Cai Y, Chen X. Research progress on the regulation of oxidative stress by phenolics: the role of gut microbiota and Nrf2 signaling pathway. J Sci Food Agric. 2024;104(4):1861–1873.
33. Miranda DG, Tomé FM, Miguel MMV, Liberato SFS, Marcucci MC, Vigerelli H, Rodrigues FP, Pacheco-Soares C, Godoi BH, Carrouel F. *Gymnema sylvestre* as a Potential Anti-Inflammatory and Anti-Biofilm Agent Against Anaerobic Infections: An In Vitro Study. Plants, 2025;14(4):497.
34. Kelleher SL, Burkinshaw S, Kuyooro SE. Polyphenols and lactation: Molecular evidence to support the use of botanical galactagogues. Mol Nutri Food Res. 2024;68(9):2300703.
35. Satria D, Hanafiah OA, Pertiwi D, Waruwu SB, Kaban VE. ADMET properties, antioxidant activity and determination of Artocarpin level in hydroalcohol extract of *Artocarpus lakoocha* Buch. Ham leaves. Res J Pharm Technol. 2025;18(7):3073–3079.
36. Rodríguez B, Pacheco L, Bernal I, Piña M. Mechanisms of action of flavonoids: antioxidant, antibacterial and antifungal properties. Ciencia Ambiente y Clima. 2023;6(2):33–66.
37. Tumilaar SG, Hardianto A, Dohi H, Kurnia D. A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global

perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. J Chem. 2024;2024(1):5594386.
38. Rapozzi V, Xodo L, Bonavida B. Therapeutic Applications of Nitric Oxide in Cancer and Inflammatory Disorders: Third International Conference on Therapeutic Application of Nitric Oxide in Cancer and Inflammatory Disorders. Elsevier; 2024.
39. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sarifi-Rad J. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023;11:1158198.
40. Keerthika K and Raghu S. Efficacy of *Gymnema Sylvestre* as a Potent Antioxidant: An In Vitro Study. Ann Med Health Sci Res. 2021;11:232-236
41. Febin KP, Syamjith P, Jothi DET. Review on Anti-Oxidant Potential of *Gymnema sylvestre*. Int J Pharm Sci. 2025;3(3):1682-1692.
42. Jee SB, Sawal A. Physiological changes in pregnant women due to hormonal changes. Cureus. 2024;16(3):e55544.
43. Zahara IA, Ulfa SM, Safitri A. Comparative Analysis of Different Natural Polymers as Coating Agents for Freeze-Dried Microencapsulation of *Cosmos caudatus* Kunth Compounds. Sci World J. 2024;2024(1):6833341.
44. Qi-Lytle X, Sayers S, Wagner EJ. Current review of the function and regulation of tuberoinfundibular dopamine neurons. Inter J Mol Sci. 2023;25(1):110.
45. Mukherjee J, Das PK, Banerjee D. Lactation physiology. In: Textbook of veterinary physiology. Springer; 2023. p. 639–674.
46. Hidayati D, Syafar M, Syamsuddin S, Jusuf EC, Ahmad M, Chaliks R. (2025). Association of oxytocin massage with oxytocin hormone levels and breast milk production in mothers with postpartum blues. Med J Malays. 2025;80(6):848–852.
47. Jiang H, Mi X, Zhu Z, Li X, Zhang Y, Liu J. Mechanisms and regulation of mammary gland development in dairy animals. Animal Advances. 2025;2:e025.
48. Rea JJ, Liu CM, Hayes AMR, Ohan R, Schwartz GM, Bashaw AG, Klug ME, Decarie-Spain L, Park Y, Kao AE, Grinevich V, Kanoski SE. Oxytocin neurons in the paraventricular and supraoptic hypothalamic nuclei bidirectionally modulate food intake. Mol Metab. 2025;102220.
49. Lacasse P. Interactions between prolactin and local regulation of the mammary gland. J Dairy Sci. 2025;108(7):6587-6600.
50. Allen-Gregory E, Pence J, Schallert MC, Bloomer RJ. An Overview of Breastfeeding and the use of Nutraceuticals and Lifestyle Factors to Support Milk Production. J Food Nutr Sci. 2025;6(1):30-54.
51. Amato S, Averna M, Guidolin D, Ceccoli C, Gatta E, Candiani S, Pedrazzi M, Capraro M, Maura G, Agnati LF, Cervetto C, Marcoli M. Heteromerization of Dopamine D2 and Oxytocin Receptor in Adult Striatal Astrocytes. Int J Mol Sci. 2023;24(5):4677.
52. Emberesh RA, Alrawi QKJ. Prolactin Hormone. Grn Int J Apl Med Sci. 2024;2(2):64-74.
53. Standing D, Dandawate P, Anant S. Prolactin receptor signaling: A novel target for cancer treatment-Exploring anti-PRLR signaling strategies. Front Endocrinol (Lausanne). 2023;13:1112987.
54. Obaid NH. A statistical study of the high prolactin hormone and its relationship to male and female infertility in the Najaf district. College of Pure Science, University of Kerbala; 2024.
55. Hidalgo-Figueroa M, Salazar A, Romero-López-Alberca C, MacDowell KS, García-Bueno B, Bioque M, Bernardo M, Parellada M, González-Pinto A, García-Portilla MP, Lobo A. Association of prolactin, oxytocin, and homocysteine with the clinical and cognitive features of a first episode of psychosis over a 1-year follow-up. Int J Neuropsychopharmacol. 2023;26(11):796-807.
56. Hannan FM, Elajnaf T, Vandenberg LN, Kennedy SH, Thakker R V. Hormonal regulation of mammary gland development and lactation. Nat Rev Endocrinol. 2023;19(1):46–61.
57. Deeh PB, Kim HY, Han K, Sathiyaseelan A, Cho HJ, Wang MH. A Novel Polyherbal Formulation Modulates Cyclophosphamide-Induced Cytotoxicity in TM3 Leydig Cells and Delays Fictive Ejaculation in Spinal Cord Transected Male Rats. Pharmaceuticals. 2025;18(6):803.
58. Zenaw A, Chane E, Fetene G, Admas S, Alemu N, Tigabie M, Biadgo B, Worede A. Serum prolactin level in male type 2 diabetes mellitus patients at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, 2024. Sci Rep. 2025.
59. Bocianska K, Bochynski H, Markwitz M, Katulski K. Prolactin's diverse physiological roles and the clinical significance of hyperprolactinemia. Ginekol Pol. 2025;96(9):787-792.
60. Buckley S, Uvnäs-Moberg K, Pajalic Z, Luegmair K, Ekström-Bergström A, Dencker A, Massarotti C, Kotlowska A, Callaway L, Morano S, Olza I. Maternal and newborn plasma oxytocin levels in response to maternal synthetic oxytocin administration during labour, birth and postpartum–a systematic review with implications for the function of the oxytocinergic system. BMC pregnancy childbirth. 2023;23(1):137.
61. Pahuja A, Jain M, Rawat K. A Review on Galactogogic Properties of India's Rich Tradition of Medicinal Herbs and Spices for Lactation. Trad Integr Med. 2024;9(3):308-317.
62. Kusumahati E, Kintoko, Sagiran. Exploration of the Function of Galactagogue in Fenugreek, Moringa, and *Sauropus Androgynus* Polyherbal Tea: Systematic Literature Review. Side: Scientific Development Journal. 2025;2(6):233–247.
63. Chowdhury R, Bhuia MS, Rakib AI, Hasan R, Coutinho HDM, Araújo IM, Menezes IRAD, Islam MT. Assessment of quercetin antiemetic properties: in vivo and in silico investigations on receptor binding affinity and synergistic effects. Plants. 2023;12(24):4189.
64. Petersenn S, Fleseriu M, Casanueva FF, Giustina A, Biermasz N, Biller BMK, Bronstein M, Chanson P, Fukuoka H, Gadelha M, Greenman Y, Gurnell M, Ho KKY, Honegger J, Ioachimescu AG, Kaiser UB, Karavitaki N, Katznelson L, Lodish M, Maiter D, Marcus HJ, McCormack A, Molitch M, Muir CA, Neggers S, Pereira AM, Pivonello R, Post K, Raverot G, Salvatori R, Samson SL, Shimon I, Spencer-Segal J, Vila G, Wass J, Melmed S. Diagnosis and management of prolactin-secreting pituitary adenomas: a Pituitary Society international Consensus Statement. Nat Rev Endocrinol. 2023;19(12):722–740.
65. Salgueiro WG, Soares MV, Martins CF, Paula FR, Rios-Anjos RM, Carrazoni T, Mori MA, Müller RU, Aschner M, Dal Belo CA, Ávila DS. Dopaminergic modulation by quercetin: *In silico* and *in vivo* evidence using Caenorhabditis elegans as a model. Chem Biol Interact. 2023;382:110610.
66. Kiyama R. Estrogenic flavonoids and their molecular mechanisms of action. J Nutr Biochem. 2023;114:109250.