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Phytochemical Characterization, Antioxidant Activity, Total Phenolic Content and Potential Fertility-Enhancing Effects of *Zingiber officinale* Roscoe Rhizome Extract

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ABSTRACT

Background and Objective: *Zingiber officinale* Roscoe (ginger), a member of the family Zingiberaceae, is an important medicinal and culinary plant with a long history of traditional use. Its rhizome contains a diverse range of biologically active constituents, including phenolic compounds, gingerols, shogaols, paradols, zingerone, flavonoids, terpenoids and volatile oils. These constituents have been associated with various biological and pharmacological activities, including antioxidant, anti-inflammatory and antimicrobial effects. Oxidative stress is also recognized as an important factor contributing to impaired reproductive function, partly through oxidative damage to spermatozoa and reproductive tissues. The present study aimed to characterize the qualitative phytochemical profile and evaluate the antioxidant activity and total phenolic content of *Z. officinale* rhizome extract. In addition, the potential fertility-enhancing significance of the extract was discussed in relation to its observed phytochemical and antioxidant properties and the available experimental and clinical evidence.

Materials and Methods: Fresh rhizomes of *Z. officinale* were collected from a local market in Karjat, Maharashtra, India. The rhizomes were washed, shade-dried, powdered and extracted with petroleum ether using a Soxhlet apparatus for 72 hrs. Qualitative phytochemical screening was conducted using standard procedures. Antioxidant activity was evaluated using the DPPH free-radical scavenging assay, whereas total phenolic content was determined using the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g of extract. All quantitative measurements were performed in triplicate.

Results: Qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, tannins, proteins, steroids, glycosides, terpenoids and saponins. The DPPH assay demonstrated a radical-scavenging inhibition of 60.00±0.25%. The total phenolic content was 3.27±0.05 mg GAE/g of extract. The detection of phenolic and flavonoid constituents, together with the observed antioxidant activity, suggests that the extract possesses antioxidant potential that may contribute to its ability to counteract oxidative stress. Previous experimental studies have reported beneficial effects of ginger on sperm quality, testosterone concentrations, oxidative stress and other reproductive parameters. In addition, a randomized clinical study has reported a reduction in sperm DNA fragmentation following ginger supplementation.

Conclusion: The present findings demonstrate that *Z. officinale* rhizome extract contains a diverse range of phytochemical constituents and exhibits appreciable antioxidant activity. The observed phenolic content and DPPH radical-scavenging activity, considered together with previously published evidence regarding the reproductive effects of ginger, support the potential fertility-enhancing significance of the extract, particularly in the context of oxidative-stress-related reproductive impairment. However, the present study did not directly assess fertility, sperm parameters, reproductive hormone concentrations, or histopathological changes in reproductive tissues. Therefore, the findings should not be interpreted as direct evidence of fertility-enhancing efficacy. Further well-designed in vivo and clinical studies are warranted to establish the direct reproductive effects, dose-response relationship, underlying mechanisms of action and safety profile of the extract.

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INTRODUCTION

Medicinal plants have played an important role in traditional healthcare systems and continue to serve as valuable sources of biologically active compounds for pharmaceutical, nutraceutical and functional food applications. Plant-derived secondary metabolites, such as phenolic compounds, flavonoids, alkaloids, tannins, terpenoids, glycosides and saponins, are associated with a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory and protective effects[1-4].

Oxidative stress occurs when the production of Reactive Oxygen Species (ROS) exceeds the capacity of endogenous antioxidant defense mechanisms. Excessive ROS can induce the oxidation of lipids, proteins and nucleic acids and may consequently impair cellular structure and function[5,6]. Reproductive tissues are particularly susceptible to oxidative damage because spermatozoa contain a high proportion of polyunsaturated fatty acids and have relatively limited cytoplasmic antioxidant defenses. Excessive oxidative stress may therefore contribute to lipid peroxidation, mitochondrial dysfunction, sperm DNA damage and impaired sperm motility and viability[7,8].

The search for natural antioxidants has consequently received considerable attention. Phenolic compounds and flavonoids obtained from medicinal plants can donate hydrogen atoms or electrons to reactive radicals and may thereby interrupt oxidative chain reactions. The antioxidant properties of plant extracts are frequently evaluated using in vitro assays such as DPPH radical-scavenging, ABTS, ferric reducing antioxidant power and lipid-peroxidation assays[5,9]. Total phenolic content is also commonly estimated using the Folin–Ciocalteu method, with results generally expressed as gallic acid equivalents[9]. However, phenolic content and antioxidant activity may vary according to plant material, cultivar, geographical origin, maturity, processing conditions, extraction solvent, extraction temperature and analytical procedure[10,11].

Zingiber officinale Roscoe, commonly known as ginger, is an herbaceous perennial plant belonging to the family Zingiberaceae. The rhizome is widely consumed as a spice and has been used traditionally in several systems of medicine for digestive disorders, nausea, vomiting, inflammatory conditions, respiratory complaints and other ailments[1,12,13]. Its extensive traditional use has stimulated considerable scientific interest in its phytochemical composition and pharmacological activities. Modern pharmacological investigations have reported antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer and neuroprotective activities associated with ginger and its constituents[2-4,12].

The rhizome of ginger contains a complex mixture of volatile and non-volatile constituents. Major non-volatile pungent phenolic compounds include gingerols, shogaols, paradols, zingerone and related diarylheptanoids, whereas volatile oils contain sesquiterpenes and monoterpenes[12-16]. Among these, 6-gingerol is one of the principal pungent constituents of fresh ginger, while dehydration of gingerols during drying or heating can result in the formation of shogaols[13,14,17]. Thus, processing can alter the relative abundance of major pungent constituents and may influence the biological properties of ginger extracts. Mao et al.[2] highlighted the importance of gingerols, shogaols and other bioactive compounds in the antioxidant and pharmacological activities of ginger, while El Sheikha et al.[4] emphasized its phytochemical complexity and potential applications in functional foods. Ginger essential oil represents another important fraction of the rhizome and contains numerous volatile constituents that contribute to its overall biological profile[15].

Several studies have demonstrated the antioxidant activity of ginger extracts and isolated ginger constituents. Gingerols and shogaols can scavenge free radicals and inhibit oxidative processes, while other phytochemicals may contribute synergistically to the overall antioxidant capacity of the extract[2,10,17-19]. Si et al.[19] demonstrated antioxidant properties of ginger extract and its major constituents, including 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol and highlighted the contribution of their chemical structures to antioxidant activity. Similarly, studies evaluating ginger rhizome extracts have reported measurable total phenolic content and antioxidant activity[10,11]. These findings indicate that the antioxidant potential of ginger may be related, at least in part, to its phenolic constituents, although differences in extraction and analytical procedures can influence the measured values.

In addition to antioxidant activity, increasing experimental evidence suggests that ginger may have beneficial effects on male reproductive function. Oxidative stress is an important factor in impaired sperm function and antioxidant-rich plant extracts may help protect spermatozoa and testicular tissues against oxidative injury[7,8,20–24]. Experimental studies have reported improvements in sperm motility, viability, testosterone levels, testicular function and other reproductive parameters following ginger administration[20–24]. Khaki et al.[20] reported beneficial effects of ginger administration on sperm-related parameters and antioxidant status in rats, while Kamtchouing et al.[21] investigated the androgenic activity of *Z. officinale* and reported effects relevant to male reproductive function. In experimental models of diabetes-associated reproductive dysfunction, ginger administration has also been associated with improvements in fertility-related parameters, sperm count, sperm motility, serum testosterone and antioxidant status[22,23]. Akinyemi et al.[24] further reported improved reproductive function following dietary supplementation with ginger and turmeric in hypertensive male rats.

Systematic reviews have summarized the available experimental evidence and suggested that ginger may improve several sperm-related parameters, including sperm concentration, viability, motility, morphology and DNA

integrity, potentially through antioxidant and androgenic mechanisms[7]. Banihani[25] similarly reviewed the available evidence concerning ginger and semen quality but emphasized the need for additional human studies. Importantly, evidence in humans remains limited. A double-blind randomized placebo-controlled clinical trial involving 100 infertile men reported a significant reduction in sperm DNA fragmentation following three months of ginger supplementation, although significant differences in conventional semen parameters were not observed[8]. Thus, the available evidence supports a potential reproductive-protective role of ginger but does not establish definitive clinical fertility-enhancing efficacy.

Despite the growing evidence concerning the biological activities of ginger, further characterization of its phytochemical composition, antioxidant activity and total phenolic content remains relevant because these properties can vary substantially according to plant material and extraction conditions. Moreover, although experimental and limited clinical evidence suggests possible reproductive-protective effects, the relationship between the phytochemical and antioxidant characteristics of ginger and its potential reproductive significance requires cautious interpretation. *In vitro* antioxidant activity alone cannot establish fertility-enhancing efficacy.

The present study was therefore designed to characterize the qualitative phytochemical constituents, evaluate the in vitro antioxidant activity and determine the total phenolic content of *Z. officinale* rhizome extract. In addition, the potential fertility-enhancing significance of the extract was considered in the context of its antioxidant phytochemical profile and previously published experimental and clinical evidence.

MATERIALS AND METHODS

Collection of plant material: Fresh rhizomes of *Zingiber officinale* Roscoe were purchased from a local market in Karjat, Maharashtra, India. The plant material was identified and authenticated based on standard botanical characteristics (Fig. 1).

Processing of plant material: The collected rhizomes were washed thoroughly with water to remove adhering soil and other impurities. The rhizomes were cut into small pieces and shade-dried at room temperature until completely dry. The dried material was powdered using a laboratory grinder and stored in airtight containers until extraction.

Soxhlet extraction: The powdered rhizome material was subjected to continuous Soxhlet extraction using petroleum ether as the extraction solvent for 72 hrs. Soxhlet extraction provides continuous contact between the plant material and solvent and is a commonly used conventional extraction technique for plant-derived constituents[16] (Fig. 2).

Fig. 1: Fresh rhizomes of *Zingiber officinale* used for the study

Fig. 2: Shade drying and powder preparation of *Zingiber officinale* rhizomes.

Fig. 3: Soxhlet extraction apparatus used for extraction of *Zingiber officinale* rhizome powder.

After completion of the extraction, the extract was filtered and concentrated using a rotary evaporator. The concentrated extract was stored under appropriate conditions and used for subsequent phytochemical and antioxidant analyses (Fig. 3).

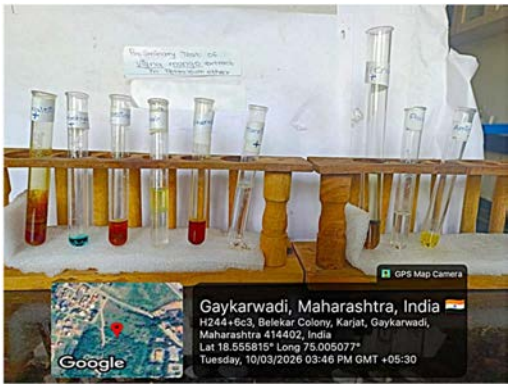

Fig. 4: Qualitative phytochemical screening of *Zingiber officinale* rhizome extract

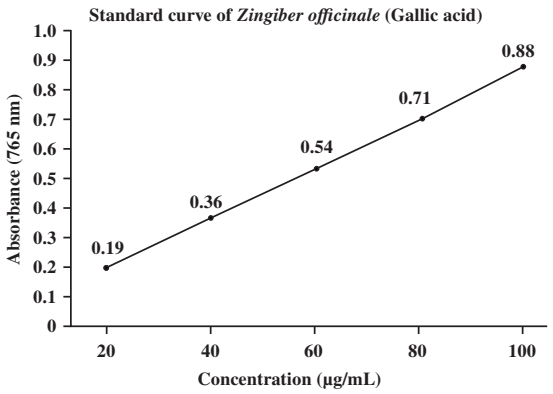

Fig. 5: Gallic acid calibration curve used for estimation of total phenolic content

Qualitative phytochemical screening: The extract was subjected to qualitative phytochemical screening using standard procedures[5]. Tests were performed for the detection of alkaloids, carbohydrates, flavonoids, phenols, tannins, proteins, steroids, glycosides, terpenoids and saponins.

The observations were recorded based on characteristic colour development, precipitate formation, or foam formation (Fig. 4).

DPPH radical-scavenging assay: The free radical-scavenging activity of the extract was evaluated using the DPPH method described by Brand-Williams et al.[26] with appropriate modifications. The DPPH is a stable nitrogen-centered free radical and its reduction by antioxidant compounds results in a decrease in absorbance. The absorbance was measured at 517 nm using a UV-visible spectrophotometer. The percentage inhibition was calculated using the following equation. The assay was performed in triplicate.

$$\text{DPPH inhibition (\%)} = \frac{\text{Control absorbance - sample absorbance}}{\text{Control absorbance}} \times 100$$

Estimation of total phenolic content: Total phenolic content was estimated using the Folin-Ciocalteu method[9]. Gallic acid was used as the reference standard. The absorbance was recorded at 765 nm. The results were expressed as mg gallic acid equivalents (GAE)/g extract. All measurements were carried out in triplicate (Fig. 5).

Literature-based interpretation of potential reproductive significance: The fertility-enhancing potential discussed in the present study was assessed from the perspective of the observed antioxidant and phytochemical properties and previously published experimental and clinical evidence. Studies reporting the effects of ginger on sperm parameters, testosterone levels, oxidative stress, reproductive tissues and sperm DNA integrity were considered[7,8,20–25].

No direct fertility experiment, animal reproductive study, semen analysis, or hormonal assessment was performed in the present investigation. Therefore, the term *fertility-enhancing potential* is used to indicate a possible reproductive-protective effect supported by the literature rather than experimentally confirmed fertility enhancement.

RESULTS AND DISCUSSION

Qualitative phytochemical screening: The qualitative phytochemical screening of *Z. officinale* rhizome extract revealed the presence of several classes of secondary metabolites. Table 1 shows the qualitative phytochemical profile of Zingiber officinale rhizome extract. The results indicate that the petroleum ether rhizome extract contained a broad range of detectable phytochemical classes. The presence of phenols and flavonoids is particularly relevant to the antioxidant activity observed in the present investigation.

Dpph radical-scavenging activity: The DPPH assay demonstrated considerable radical-scavenging activity of the extract. The extract showed 60.00±0.25% DPPH radical-scavenging inhibition, indicating appreciable *in vitro* antioxidant activity.

The observed antioxidant activity is consistent with previous reports describing the radical-scavenging properties of ginger extracts and ginger-derived phenolic constituents (Table 2, Fig. 6)[2,10,17,19].

Total phenolic content: The total phenolic content of the extract was estimated using the Folin-Ciocalteu method. The presence of phenolic compounds may contribute to the antioxidant capacity of the extract because phenolics can act as hydrogen donors and reducing agents (Table 3 and Fig. 7)[9-11,19].

The present study demonstrated that *Z. officinale* rhizome extract contains a diverse range of phytochemical constituents, including alkaloids, carbohydrates, flavonoids, phenols, tannins, proteins, steroids, glycosides, terpenoids

Fig. 6. DPPH radical-scavenging assay of *Zingiber officinale* rhizome extract

Fig. 7: Total phenolic content estimation of *Zingiber officinale* rhizome extract using the Folin–Ciocalteu method

Table 1: Qualitative phytochemical profile of *Zingiber officinale* rhizome extract

Phytochemical constituent	Test performed	Observation	Results
Alkaloids	Mayer's test	Cream precipitate	+
Carbohydrates	Molisch's test	Violet ring	+
Flavonoids	Alkaline reagent test	Yellow coloration	+
Phenols	Ferric chloride test	Blue-green colour	+
Tannins	Ferric chloride test	Dark green colour	+
Proteins	Biuret test	Violet coloration	+
Steroids	Salkowski test	Reddish-brown ring	+
Glycosides	Keller-Kiliani test	Brown ring	+
Terpenoids	Salkowski test	Reddish-brown coloration	+
Saponins	Foam test	Stable foam	+

+: Detected

Table 2: DPPH radical-scavenging activity of *Zingiber officinale* rhizome extract

Replicate	Control absorbance	Sample absorbance	Inhibition (%)
R1	0.700	0.281	59.86
R2	0.700	0.278	60.29
R3	0.700	0.281	59.86
Mean±SD	—	—	60.00±0.25

Table 3: Total phenolic content of *Zingiber officinale* rhizome extract

Replicate	Absorbance	TPC (mg GAE/g extract)
Replicate 1	0.58	3.22
Replicate 2	0.60	3.31
Replicate 3	0.59	3.28
Mean±SD	0.59±0.01	3.27±0.05

The total phenolic content was therefore (TPC) = 3.27±0.05 mg GAE/g extract

and saponins. The phytochemical diversity observed in the present investigation is broadly consistent with previous reports describing ginger as a chemically complex medicinal plant containing phenolic compounds, gingerols, shogaols, flavonoids, terpenoids and essential oils[2-4,12].

The presence of phenolic compounds and flavonoids is particularly relevant because these compounds are widely recognized for their antioxidant properties. Phenolic molecules may neutralize free radicals through hydrogen donation or electron transfer and can thereby interrupt oxidative chain reactions[5,9,17].

The DPPH assay demonstrated 60.00±0.25% radical-scavenging activity. The DPPH is a widely used *in vitro* method for the preliminary assessment of free-radical-scavenging capacity[26,27]. The observed activity suggests that the extract contains constituents capable of reducing the DPPH radical under the experimental conditions used.

The antioxidant activity observed in the present study is consistent with previous research on ginger. Si et al.[19] demonstrated that ginger extract and isolated gingerols and shogaols possess antioxidant properties. Similarly, studies investigating ginger rhizome extracts have reported antioxidant activity associated with phenolic and other bioactive constituents[10,11].

The total phenolic content of the present extract was 3.27±0.05 mg GAE/g extract. Phenolic content is influenced by plant genotype, geographical origin, maturity, drying procedure, extraction solvent and analytical methodology[3,4,11]. Consequently, differences between the value observed in the present study and those reported by other researchers may be expected.

The antioxidant properties of ginger have been associated particularly with gingerols and shogaols. 6-Gingerol is a major pungent phenolic compound of fresh ginger, whereas 6-shogaol is generated through dehydration reactions and may become more abundant during drying and heating[13,17]. Both compounds possess antioxidant and anti-inflammatory properties[16,17,19].

The observed antioxidant activity is also relevant to reproductive health. Oxidative stress can adversely affect sperm membranes, mitochondrial activity and DNA integrity. Because spermatozoa are particularly susceptible to oxidative damage, antioxidant protection is considered an important component of reproductive health[7,8].

Experimental evidence supports a possible relationship between ginger administration and improved reproductive parameters. Khaki et al.[20] reported beneficial effects of ginger on spermatogenesis and sperm parameters in rats. Kamtchouing et al.[21] reported androgenic activity of *Z. officinale* in male rats, suggesting that ginger constituents may influence reproductive endocrine function.

Shalaby and Hamowieh[22] investigated ginger extracts in diabetic male rats and observed improvements in fertility index, sperm count, sperm motility and serum testosterone. Ghlissi et al.[23] also reported antioxidant and androgenic effects of dietary ginger on reproductive function in diabetic rats. These findings are relevant because diabetes-associated oxidative stress can impair male reproductive function.

Akinyemi et al.[24] demonstrated that dietary supplementation with ginger and turmeric improved reproductive function in hypertensive male rats. Although, the combined use of ginger and turmeric prevents attribution of the observed effects exclusively to ginger, the study provides additional evidence for the possible role of antioxidant-rich plant interventions in reproductive protection.

The antioxidant mechanism provides a plausible explanation for some of these reproductive effects. Ginger-derived phenolics may reduce oxidative stress and protect cellular membranes and DNA from oxidative damage. In addition, ginger has been associated with modulation of androgenic and endocrine pathways in experimental models[21-24].

Human evidence, however, must be interpreted cautiously. Hosseini et al.[8] conducted a randomized, double-blind, placebo-controlled trial in 100 infertile men and reported a significant reduction in sperm DNA fragmentation after three months of ginger supplementation. Importantly, no significant differences were observed in conventional semen parameters between groups[8]. Therefore, ginger should not currently be described as a clinically established fertility treatment.

Banihani[25] reviewed experimental evidence concerning ginger and semen quality and reported beneficial effects on sperm concentration, viability, motility and morphology in several experimental studies, while emphasizing the need for additional human evidence. Similarly, Gholami-Ahangaran et al.[7] concluded from a systematic review that ginger may improve several sperm parameters through antioxidant and androgenic mechanisms.

The potential reproductive significance of *Zingiber officinale* in the present study may be related to its phytochemical composition and antioxidant activity. The presence of phenolic compounds, flavonoids, gingerols and shogaols, together with the observed DPPH radical-scavenging activity (60%) and total phenolic content (3.27 mg GAE/g extract), indicates that the extract contains antioxidant-active constituents that could potentially contribute to protection against oxidative stress. A proposed mechanism illustrating the possible role of these bioactive compounds in reproductive protection and fertility-related outcomes is presented in Fig. 8.

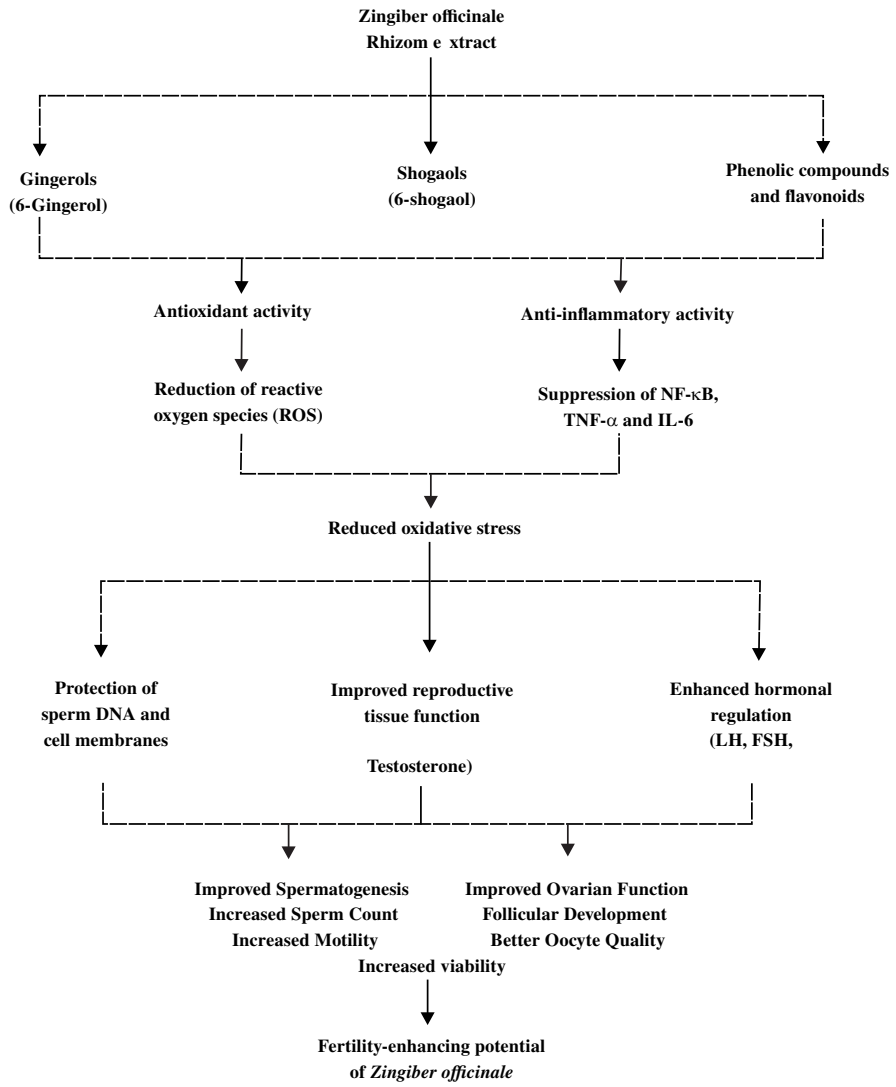

Fig. 8: Proposed mechanism of fertility-enhancing potential of *Zingiber officinale* rhizome extract

The present findings therefore provide a biochemical basis for considering the potential reproductive-protective significance of ginger, particularly through antioxidant mechanisms. The observed phenolic content and DPPH activity indicate that the extract contains antioxidant-active constituents. However, the present investigation did not measure sperm count, sperm motility, sperm morphology, testosterone, LH, FSH, sperm DNA fragmentation, or pregnancy outcomes.

Consequently, the present study cannot establish direct fertility-enhancing efficacy. Rather, it provides preliminary phytochemical and antioxidant evidence that supports further investigation of ginger as a potential reproductive-health-supporting plant.

Fertility-enhancing potential of *Zingiber officinale*

Relationship between oxidative stress and fertility: Oxidative stress is an important factor in male reproductive dysfunction. Excessive ROS can damage sperm membrane lipids, mitochondrial structures and nuclear DNA, potentially reducing sperm motility, viability and fertilization competence[7,8].

The antioxidant activity observed in the present *Z. officinale* extract may therefore be relevant to reproductive protection. Phenolic compounds can neutralize reactive species and reduce oxidative damage, while gingerols and shogaols have demonstrated antioxidant properties[16,17,19].

Potential effects on male reproductive function: Several animal studies have reported beneficial effects of ginger on male reproductive parameters. Khaki et al.[20] observed improvements in sperm parameters following ginger administration in rats. Kamtchouing et al.[21] demonstrated androgenic activity of ginger in male rats.

Shalaby and Hamowieh[22] reported increased sperm count, sperm motility, testosterone levels and fertility

index in diabetic male rats treated with ginger extracts. Ghlissi et al.[23] reported antioxidant and androgenic effects of dietary ginger in diabetic rats.

These findings suggest that ginger may influence reproductive function through a combination of antioxidant and endocrine mechanisms.

Potential protection of sperm DNA: Sperm DNA integrity is an important factor in reproductive success. Oxidative stress can cause sperm DNA fragmentation and may negatively influence reproductive outcomes.

The clinical study by Hosseini et al.[8] is particularly relevant because ginger supplementation was associated with a reduction in sperm DNA fragmentation in infertile men. However, because conventional semen parameters were not significantly improved, these findings should be interpreted as evidence of a possible protective effect rather than definitive evidence of increased fertility.

Role of phenolic compounds: The total phenolic content of the present extract was 3.27±0.05 mg GAE/g extract. Phenolic compounds can function as antioxidants by donating hydrogen atoms or electrons to free radicals[6,9].

Gingerols, shogaols and related phenolic compounds may therefore contribute to the potential reproductive-protective effects of ginger by limiting oxidative damage to reproductive cells[16,17,19].

Possible mechanisms: Based on the present findings and published evidence, the possible mechanisms underlying the reproductive-protective and fertility-related effects of ginger may include:

- Reduction of oxidative stress in reproductive tissues[7,20].
- Protection of sperm membrane integrity against lipid peroxidation[7,19]
- Reduction of oxidative damage to sperm DNA[8]
- Improvement of sperm motility and viability observed in experimental models[7,20,22]
- Possible enhancement of testosterone levels and androgenic activity[21–23]
- Modulation of antioxidant defense mechanisms[7,23]
- Protection of testicular tissue against oxidative injury[22–24]
- Possible interaction with inflammatory and cellular signaling pathways[2,16,17]

These mechanisms remain hypothetical for the specific extract examined in the present study and require direct experimental confirmation.

Bioactive phytochemicals including gingerols, shogaols, flavonoids and phenolic compounds exert antioxidant and anti-inflammatory effects, potentially contributing to the reduction of oxidative stress, protection of reproductive tissues, modulation of reproductive hormone balance and improvement of male and female fertility-related parameters.

Pharmacological significance

Antioxidant potential: The 60.00±0.25% DPPH inhibition observed in the present study indicates appreciable antioxidant activity. Ginger phenolics, particularly gingerols and shogaols, are recognized contributors to its antioxidant properties[2,16,17,19].

Anti-inflammatory potential: Gingerols and shogaols have been associated with the modulation of inflammatory signaling pathways. Reviews have described effects involving pathways such as NF-κB and other inflammatory mediators[2,16,17].

Antimicrobial potential: Ginger essential oils and phenolic constituents have demonstrated antimicrobial activity against several microorganisms[12,15]. These properties further contribute to the pharmacological importance of the plant.

Anticancer potential: Experimental studies have reported anticancer effects associated with gingerols and shogaols, including the modulation of apoptosis, oxidative stress and inflammatory signaling[2,17]. These observations support continued investigation of ginger-derived compounds in pharmacological research.

Reproductive and fertility-related potential: The reproductive significance of ginger is supported mainly by experimental evidence. Animal studies have reported improvements in sperm parameters, testosterone levels and fertility indices[20-24]. Human evidence is currently more limited, although a randomized clinical study reported reduced sperm DNA fragmentation after ginger supplementation[8].

Therefore, the reproductive potential of ginger should presently be regarded as promising but not clinically established.

Toxicological and safety considerations: Ginger has a long history of dietary and traditional medicinal use and is generally considered to have a favorable safety profile when consumed at customary levels[1,13]. Experimental investigations have also reported relatively favorable safety profiles for ginger extracts[22].

However, safety depends on dose, extraction method, preparation, duration of exposure and individual physiological conditions. High-dose experimental exposure may produce adverse effects and reproductive or pregnancy-related applications require particular caution. Recent evidence indicates that dose and preparation are important determinants of safety in reproductive and developmental contexts[3,4].

Therefore, standardization of the extract, identification of active compounds, dose-response studies and comprehensive toxicological evaluation are required before therapeutic fertility-related applications can be proposed.

Limitations of the present study: The present study has several limitations:

- Only qualitative phytochemical screening was performed; individual compounds such as 6-gingerol and 6-shogaol were not quantified
- The extract was prepared using petroleum ether; therefore, the observed phytochemical profile represents only the constituents extracted under these conditions
- Antioxidant activity was evaluated using only the DPPH assay
- No IC50 value was determined
- No comparative standard antioxidant was included in the reported DPPH dataset.
- Fertility-related parameters such as sperm count, motility, morphology, testosterone, LH, FSH, reproductive organ weight and sperm DNA fragmentation were not directly measured
- The fertility-enhancing potential is therefore based on interpretation of the present antioxidant findings together with previously published literature

Further HPLC, GC-MS, LC-MS/MS and molecular studies are required for the identification and quantification of specific bioactive compounds.

Future perspectives: Future studies should focus on the quantitative characterization of ginger bioactive constituents using HPLC, HPTLC, GC-MS and LC-MS/MS. Particular attention should be given to 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, zingerone and related phenolic compounds[13,16,17].

Further antioxidant studies should include DPPH, ABTS, FRAP, nitric oxide scavenging and lipid-peroxidation assays. *In vivo* studies should investigate sperm count, motility, morphology, sperm DNA integrity, testosterone, LH, FSH, testicular histology and fertility indices.

Mechanistic investigations involving oxidative-stress markers, antioxidant enzymes, inflammatory pathways and reproductive signaling pathways may help establish the relationship between ginger phytochemicals and reproductive function.

Well-designed clinical trials are also necessary to determine whether the promising experimental effects of ginger translate into meaningful improvements in human reproductive outcomes[7,8,25].

CONCLUSION

The present study demonstrated that *Zingiber officinale* Roscoe rhizome extract contains a diverse range of phytochemical constituents, including alkaloids, carbohydrates, flavonoids, phenols, tannins, proteins, steroids, glycosides, terpenoids and saponins. The extract exhibited 60.00±0.25% DPPH radical-scavenging activity, indicating appreciable *in vitro* antioxidant potential. The total phenolic content was 3.27±0.05 mg GAE/g extract.

The presence of phenolic compounds and flavonoids, together with measurable antioxidant activity, provides a plausible biochemical basis for the potential protective effects of ginger against oxidative stress. Published experimental studies further indicate that ginger may improve sperm parameters, testosterone levels, antioxidant status and other reproductive indices.

The available human evidence is more limited but provides encouraging observations, particularly regarding the reduction of sperm DNA fragmentation following ginger supplementation[8]. Thus, the antioxidant and phytochemical characteristics observed in the present study, together with existing experimental evidence, support the potential reproductive-protective and fertility-related significance of *Z. officinale*.

Nevertheless, the present study did not directly evaluate fertility or reproductive parameters. Therefore, the findings should not be interpreted as proof of fertility enhancement. Further standardized phytochemical characterization, in vivo reproductive studies and controlled clinical investigations are required to establish the direct efficacy, mechanisms, optimal dose and safety of *Z. officinale* rhizome extract for fertility-related applications.

The findings support the traditional use of *Zingiber officinale* as a medicinal plant and suggest its potential application as a natural reproductive-health-supporting nutraceutical. The proposed mechanistic pathway provides a scientific basis for future experimental and clinical investigations aimed at evaluating and validating its potential reproductive health benefits.

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