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Original Article

Clinical effect of *Echinacea purpurea* as an antiviral and its effect on reproductive hormones

[Efecto clínico de *Echinacea purpurea* como antiviral y su efecto sobre hormonas reproductoras]

Revi G.H. Novika[1,2*], Nurul J. Wahidah[1], Ahmad Yunus[3,4], Lanjar Sumarno[5], Muhana F. Ilyas[6,7]

[1]Midwefy Study Program, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
[2]Master Program in Public Health, Graduate School, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
[3]Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
[4]Center for Research and Development of Biotechnology and Biodiversity, LPPM Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
[5]Research Center for Agroindustry, National Research and Innovation Agency, Bogor, West Java, Indonesia.
[6]Department of Neurology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
[7]Department of Anatomy, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.
*E-mail: revi.gama@staff.uns.ac.id

Abstract

Context: *Echinacea purpurea*, a well-known herbal remedy, is believed to possess immunomodulatory properties and has been traditionally utilized for a wide range of health benefits. However, its antiviral activities, particularly against SARS-CoV-2, and its modulation of reproductive hormones remain unknown.

Aims: To investigate the utilization of *E. purpurea* herbal preparations as an antiviral by evaluating the Transmembrane Serine Protease 2 (TMPRSS2) expression and investigating its effect on reproductive hormones by measuring androgen in males and estrogen in females.

Methods: Forty male and female participants were randomly assigned to different groups. Daily administration of 400 mg of *E. purpurea* herbal preparations for 28 days constituted the intervention. The gathering of demographic data was documented. Before and after the intervention, samples were collected for investigation, which included the ELISA-based assessment of androgen, estrogen, and TMPRSS2 expression.

Results: This study indicated that administration of *E. purpurea* can significantly down-regulate TMPRSS2 expression in both males (8.39 ± 1.13 to 4.16 ± 1.53; p=0.000) and females (14.18 ± 1.93 to 5.25 ± 1.13; p=0.000). The androgen was also significantly down-regulated in the male intervention group (22.73 ± 1.75 to 12.72 ± 2.26; p=0.000). In addition, estrogen levels were also significantly up-regulated in the female intervention group (72.33 ± 11.12 to 161.14 ± 35.13; p=0.000).

Conclusions: *E. purpurea* may be capable of down-regulating androgen in males, up-regulating estrogen in females, and down-regulating TMPRSS2 expression. This study contributes to the growing body of literature exploring the effects of *E. purpurea* as an antiviral property, especially for SARS-CoV-2, and its effect on reproductive hormones.

Keywords: androgens; *Echinacea*; estrogens; reproduction.

Resumen

Contexto: Se cree que la *Echinacea purpurea*, un conocido remedio herbal, posee propiedades inmunomoduladoras y se ha utilizado tradicionalmente para una amplia gama de beneficios para la salud. Sin embargo, aún se desconocen sus actividades antivirales, en particular contra el SARS-CoV-2, y su modulación de las hormonas reproductivas.

Objetivos: Investigar la utilización de preparados herbales de *E. purpurea* como antiviral mediante la evaluación de la expresión de la proteasa transmembrana de serina 2 (TMPRSS2) e investigar su efecto sobre las hormonas reproductivas mediante la medición de andrógenos en varones y estrógenos en mujeres.

Métodos: Cuarenta participantes masculinos y femeninos fueron asignados aleatoriamente a diferentes grupos. La intervención consistió en la administración diaria de 400 mg de preparados herbales de *E. purpurea* durante 28 días. Se documentó la recogida de datos demográficos. Antes y después de la intervención, se recogieron muestras para la investigación, que incluyó la evaluación basada en ELISA de la expresión de andrógenos, estrógenos y TMPRSS2.

Resultados: Este estudio indicó que la administración de *E. purpurea* puede reducir significativamente la expresión de TMPRSS2 tanto en hombres (8,39 ± 1,13 a 4,16 ± 1,53; p=0,000) como en mujeres (14,18 ± 1,93 a 5,25 ± 1,13; p=0,000). El andrógeno también se redujo significativamente en el grupo de intervención masculino (22,73 ± 1,75 a 12,72 ± 2,26; p=0,000). Además, los niveles de estrógeno también aumentaron significativamente en el grupo de intervención femenino (72,33 ± 11,12 a 161,14 ± 35,13; p=0,000).

Conclusiones: *E. purpurea* puede ser capaz de regular a la baja los andrógenos en los varones, regular al alza los estrógenos en las mujeres y regular a la baja la expresión de TMPRSS2. Este estudio contribuye al creciente cuerpo de literatura que explora los efectos de *E. purpurea* como propiedad antiviral, especialmente para el SARS-CoV-2, y su efecto sobre las hormonas reproductivas.

Palabras Clave: andrógenos; *Echinacea*; estrógenos; reproducción.

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AUTHOR INFO
ORCID: 0000-0002-5606-835X (RGHN) 0009-0009-0759-1193 (LS)
0000-0002-6783-3299 (NJW) 0000-0002-0176-9773 (MFI)
0000-0001-6483-9671 (AY)

INTRODUCTION

Nature has always been a rich resource of therapeutic compounds, presenting us with a variety of medicinal plants that contain beneficial phytochemicals. Recently, phytotherapy has become increasingly prevalent in the treatment and prevention of a variety of medical diseases. In recent decades, there has been a worldwide increase in interest in medicinal plants that can play a role as immunomodulators, including *Echinacea purpurea*, also known as coneflower (Kumar and Ramaiah, 2011). The United States (US) Food and Drug Administration (FDA) identifies it as a food, whereas Commission E (Task Force E of the Federal Bureau of Health of Germany) authorized it as a medication. *Echinacea* is a genus of nine herbaceous, daisy-family (*Asteraceae*; *Compositae*) flowering plants indigenous to eastern and central North America. There are various applications for *Echinacea* species, components, and preparations. Three species of *Echinacea*, notably *E. purpurea*, *E. angustifolia*, and *E. pallida*, have been utilized for generations in Native American medicine. Multiple significant categories of bioactive constituents having pharmacological properties have been identified. The most important components include alkylamides, polysaccharides, glycoproteins, flavonoids, and phenolic compounds, including derivates of caffeic acid, such as caffeic acid, chicoric acid, caftaric acid, chlorogenic acid, and echinacoside, whose levels vary according to the plant's sections (Attarzadeh et al., 2020; Bruni et al., 2018). Phylloxanthobilins, 3-phellandrene, acetaldehyde, dimethyl sulfide, camphene, hexanal, apinene, and limonene were also detected in all plant tissues, regardless of species (Attarzadeh et al., 2019; Dalby-Brown et al., 2005; Kakimov et al., 2021; Maggini et al., 2019; Nyalambisa et al., 2017; Pallag et al., 2016; Ramezannezhad et al., 2019; Tabar et al., 2019; Vendramin et al., 2021).

Furthermore, current research has identified Transmembrane Serine Protease 2 (TMPRSS2) as a crucial role in viral infections, such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus disease of 2019 (COVID-19) pandemic (Iwata-Yoshikawa et al., 2022; Mahapatra et al., 2021). It has been discovered that TMPRSS2 facilitates viral entrance into host cells, emphasizing its significance in infection progression. Understanding the effect of immunomodulatory compounds, such as that of *E. purpurea,* on TMPRSS2 expression could shed light on their possible antiviral capabilities. In addition to its immunomodulatory and antiviral properties, recent research reveals that *E. purpurea* may also alter reproductive hormones. According to a study, *E. purpurea* may have an androgenic effect in male rats (Skaudickas et al., 2004). In addition, molecular docking research demonstrated that *E. purpurea* may have estrogenic effects via binding to and modulating the human estrogen receptor (Powers et al., 2015). Randomized controlled clinical trials are regarded as the gold standard for assessing the efficacy and safety of human interventions. This study aims to investigate the utilization of *E. purpurea* herbal preparations as an antiviral by evaluating the TMPRSS2 expression and investigating its effect on reproductive hormones by measuring androgen in males and estrogen in females.

MATERIAL AND METHODS

Ethical consideration

This study was reviewed and approved by The Health Research Ethics Committee Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia (reference number of 911/VI/HREC/2022 and issued on 6th July 2022). The study adhered to the Helsinki Declaration's guiding principles (World Medical Association, 2013). Before being enrolled in the study, all subjects were provided either verbal or written informed consent. The procedures and objectives of the study were communicated to the participants. In addition, participants were informed that they might withdraw from the study at any moment. This research also received support from and collaboration with the Indonesian Ministry of Education, Culture, Research and Technology.

Study locations

This is a pilot clinical trial with a posttest control group design was conducted at the Mondokan Health Center, Sragen, Central Java, Indonesia. Mondokan Health Center is one of the tertiary health centers in Indonesia where demographic data was collected, clinical evaluation and intervention administration were performed, as well as sampling, which was then used for laboratory tests at the Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. The study was conducted between May 2022 and March 2023.

Study participants

The study population comprised individuals of reproductive age residing in the Mondokan Health Center's service area in Sragen, Central Java, Indonesia. The inclusion criteria for this study consist of the following: 1) Participants including both males and females between the ages of 18 and 49 to guarantee homogeneity; 2) Subjects whose reproductive hor-

mone levels are stable within the normal range, as determined by pre-study blood tests; 3) Participants without significant chronic medical conditions or uncontrolled diseases that could impede the study's results or their ability to adopt herbal medicines. 4) Participants who are willing and able to provide written informed consent to participate in the trial, indicating that they understand the procedures, potential risks, and potential benefits of the study; 5) Participants within a specific body mass index (BMI) range (18.5-30) to remove potential confounding factors related to weight and metabolism; 6) Individuals who are not using hormone replacement therapy, oral contraceptives, or any other medications that may influence reproductive hormone levels. 7) Participants with no known allergies or hypersensitivity to *E. purpurea* or any of its components, as determined by medical history and allergy testing; and 8) Participants with the ability to adhere to the study protocol, attend scheduled visits, and complete required evaluations and questionnaires.

Exclusion criteria include those below or above the age requirements, pregnant or lactating female, those with preexisting hormonal disorders, those currently or recently using hormone-based medications, those with known allergies or hypersensitivity to *E. purpurea* or its components, and those with an autoimmune disease diagnosis. The dropout criteria include participant withdrawal, noncompliance with the study protocol, substantial adverse effects associated with herbal preparations, follow-up loss, and failure to meet exclusion criteria. These criteria guarantee that study participants are suitable for the research objectives, limit confounding variables, and maintain data integrity throughout the trial.

Sample size

Lemeshow's replication technique was used to calculate the sample size for a study (Lemeshow and David, 1997). According to the calculations, the minimum number of samples required for each group was eight. Considering a previous study's 12.5 percent failure rate (Novika et al., 2022), it was rectified using the formula 1/(1-f) x r. Therefore, the resulting sample size was 1/(1-0.125) × 8 = 9.14, rounded to 10. The researcher settled on a sample size of 10 for each group: male control group, male treatment group, female control group, and female treatment group, for a total of 40 participants.

Randomization

Randomization using Microsoft Excel was used to assign participants to distinct groups. Separate rows were created for the participant ID numbers and gender information. Using the "=RAND()" function, a person not directly involved in the study assigned random numbers to each participant in order to preserve blinding. This method ensured a fair and objective allocation process, eliminates selection bias, and enhanced the internal validity of the study.

Preparation and intervention

As a form of treatment, herbal components were utilized. Capsules of *E. purpurea* were obtained from existing products that had been approved by Indonesia's National Agency for Drug and Food Control. Instructions drawn from reliable published articles were utilized as support to estimate the dosage. In a prior trial, *E. purpurea* was provided at 400 mg per day for a total of 28 days (Li et al., 2017).

Data collection

In this study, demographic information and clinical evaluations were obtained. Age, sex, and other relevant variables were included in the demographic data. The clinical evaluations included a comprehensive assessment of the individuals' health status, including medical history and physical examinations. Before and after the intervention, blood samples from the participants were collected. The collected samples were then examined using specialized enzyme-linked immunosorbent assay (ELISA) kits. Androgen Human ELISA kit (BT-Lab, Bioassay Technology Laboratory), Cat. No. E6694Hu; Estrogen Human ELISA Kit (DEMEDITEC), Cat. No. 42K.121; and Human TMPRSS2 Human ELISA kit (BT-Lab, Bioassay Technology Laboratory), Cat. No. E0090Hu; were utilized. The analysis was undertaken according to the manufacturer's instructions supplied in each kit.

Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 27.0 (SPSS Inc. Chicago, IL, USA, 2020). Descriptive statistics analyses were generated to define the demographic characteristics of the study subjects. This involved calculating metrics, including means and standard deviations. Depending on the data distribution, either the independent t-test or the Mann-Whitney test was employed to compare demographic data. In addition, a paired student t-test or Wilcoxon signed-rank test, depending on the data distribution, was employed to assess the effect of the intervention on TPPRS2 expression and reproductive hormone levels. The significance level (alpha) was set to 0.05.

RESULTS

Characteristics of study participants

This study enrolled forty participants who met the criteria. The demographic information was observed for all participants. The overall characteristics are shown in Table 1. In this study, there were no significant differences in the entire demographic data. During study completion, none of the participants dropped out. On the first day of consumption, there were a number of adverse reactions, including nausea (n = 1) and itchiness (n = 1). The researcher gave a treatment for this reaction, and on the second day, the reaction did not occur. Researchers have also guaranteed that individuals are still willing to participate in ongoing studies.

E. purpurea on TMPPRS2 expression

This study indicated that administration of *E. purpurea* can down-regulate TMPRSS2 expression, as evidenced by a significant decrease in TMPPRS2 expression in the intervention group after 28 days of treatment in all samples (11.28 ± 3.35 to 4.70 ± 1.42 pg/mL; p=0.000) as well as both males (8.39 ± 1.13 to 4.16 ± 1.53 pg/mL; p=0.000) and females (14.18 ± 1.93 to 5.25 ± 1.13 pg/mL; p=0.005) (see Table 2).

This study also showed that administration of *E. purpurea* can also down-regulate the androgen, proven by the significant decrease in androgen levels in the intervention group after being given *E. purpurea* for 28 days in males (22.73 ± 1.75 to 12.72 ± 2.26 pg/mL; p=0.005). In addition, the administration of *E. purpurea* led to an increase in estrogen levels in females (72.33 ± 11.18 to 161.14 ± 35.13 pg/mL; p=0.000), indicating an up-regulation of estrogen (see Table 3).

DISCUSSION

E. purpurea is a well-known medicinal herb with immunomodulatory, anti-inflammatory, and antioxidant properties. *E. purpurea* has a number of bioactive compounds that have biological and pharmacological effects as an immunomodulator. These bioactive compounds are alkylamides (Cech et al., 2010; Mudge et al., 2011; Rios and Olivo, 2014; Saeidnia et al., 2015), polysaccharides (Balciunaite et al., 2015; Cai et al., 2014; Jiang et al., 2021; Mohamed Sharif et al., 2021; Xu et al., 2022; Yang et al., 2018; Yao et al., 2019), and glycoproteins (Bergeron and Gafner, 2007; Bodinet and Beuscher, 1991; Del-Rio-Navarro et al., 2006; Guiotto et al., 2008; Kim et al., 2014). Several processes are responsible for the immunostimulant effect of its formulations, including phagocytosis activation, fibroblast stimulation, and cytokine induction (Saeidnia et al., 2015; World Health Organization, 1999). It has been demonstrated that *E. purpurea* modulates both innate and adaptive immune responses (Zhai et al., 2007). By positively impacting the host's defensive mechanisms and the fate of dendritic cell production, it can activate or suppress both the innate and adaptive immune responses (El-Ashmawy et al., 2015; Haria et al., 2016; Nagoor et al., 2021). Antiviral effect also found in *Echinacea* especially in polysaccharides (Balciunaite et al., 2015; Cai et al., 2014; Jiang et al., 2021; Mohamed Sharif et al., 2021; Xu et al., 2022; Yang et al., 2018; Yao et al., 2019) and flavonoid (Agrawal, 2011; Kurkin et al., 2011).

Table 1. Characteristic of study participants.

Variable	Male				Female			
	All Group (n = 20)	Intervention group (n = 10)	Control group (n = 10)	p	All group (n = 20)	Intervention group (n = 10)	Control group (n = 10)	p
Age (years)	22.55 ± 2.625	22.80 ± 2.658	22.30 ± 2.71	0.682	23.00 ± 1.919	22.80 ± 2.150	23.20 ± 1.751	0.654
Weight (kg)	64.80 ± 4.561	64.70 ± 5.122	64.90 ± 4.202	0.925	58.40 ± 4.057	58.80 ± 2.530	58.00 ± 5.292	0.673
Height (cm)	166.35 ± 3.233	166.70 ± 3.773	166.00 ± 2.749	0.641	155.60 ± 2.664	154.90 ± 3.213	156.30 ± 1.889	0.250
BMI (kg/m^2)	23.43 ± 1.774	23.29 ± 1.792	23.58 ± 1.84	0.730	24.13 ± 1.70	24.51 ± 0.901	23.75 ± 2.235	0.339

The data is presented as the mean ± SD. The independent t-test was utilized.

Table 2. Effect of *E. purpurea* on TMPRSS2 expression (pg/mL).

Sex	Group (n)	Pre	Post	P-value
All sample (n = 40)	Intervention Group (n = 20)	11.28 ± 3.35	4.70 ± 1.42	0.000[a*]
	Control Group (n = 20)	9.48 ± 5.62	9.80 ± 4.95	0.156[a]
Male (n = 20)	Intervention Group (n = 10)	8.39 ± 1.13	4.16 ± 1.53	0.000[b*]
	Control Group (n = 10)	4.45 ± 1.83	5.31 ± 1.86	0.071[b]
Female (n = 20)	Intervention Group (n = 10)	14.18 ± 1.93	5.25 ± 1.13	0.005[a*]
	Control Group (n = 10)	14.52 ± 2.65	14.28 ± 1.85	0.823[b]

The data is presented as the mean ± SD. [a]Wilcoxon test and [b]Student paired t-test was utilized. *p<0.005, statistically significant.

Table 3. Effect of *E. purpurea* on reproductive hormone (pg/mL).

Male (androgen)				Female (estrogen)			
Group	**Pre**	**Post**	**P-value**	**Group**	**Pre**	**Post**	**P-value**
Intervention group (n = 10)	22.73 ± 1.75	12.72 ± 2.26	0.005[b*]	Intervention group (n = 10)	72.33 ± 11.18	161.14 ± 35.13	0.000[a*]
Control group (n = 10)	18.54 ± 2.86	18.93 ± 6.56	0.866[a]	Control group (n = 10)	63.96 ± 7.32	66.27 ± 7.66	0.539[a]

The data is presented as the mean ± SD . [a]Student paired t-test and [b]Wilcoxon test were utilized. *p<0.005, statistically significant.

This study shows that administration of *E. purpurea* with a dosage of 400 mg per day in 28 days can down-regulate the expression of TMPRSS2. Another study showed that treatment with *E. purpurea* extract (Echinaforce®, EF) at concentrations of 40 to 80 µg/mL resulted in significantly reduced expression of TMPRSS-2 in primary nasal epithelial cells (Vimalanathan et al., 2022). The possible mechanism is that *E. purpurea* may have the potential to inhibit interleukin-1β (IL-1β) production and reduce pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor (TNF) (Fast et al., 2015; Vieira et al., 2022), due to its components, especially polysaccharides (Roesler et al., 1991). IL-1β has been found to promote TMPRSS2 expression and SARS-CoV-2 cell entry through the p38 mitogen-activated protein kinase-GATA binding protein 2 (MAPK-GATA2) axis (Cioccarelli et al., 2021). In addition, IL-6 has been found to synergize with IL-1β and TNF to up-regulate trypsin expression, which activates matrix metalloproteinases and causes the breakdown of tissues (Gubernatorova et al., 2020; Indalao et al., 2017). A similar mechanism has been found in azithromycin, which has been found to downregulate gene expression of IL-1β and pathways involving TMPRSS2 and transmembrane protease, serine 11D gene (TMPRSS11D) (Renteria et al., 2020). The other possible mechanism is that *E. purpurea* has an antiandrogenic effect. It is well known that TMPRSS2 expression is regulated by androgens in certain cells, and androgenic receptor inhibitors such as enzalutamide can downregulate TMPRSS2 expression (Leach et al., 2021).

This study also shows that the administration of *Echinacea purpura* can down-regulate the expression of androgen in males. Other studies show similar results, in which *Echinacea* treatment for 2 to 4 weeks showed gradual antiandrogenic activity through the effect on male sexual hormone testosterone producing organ, which may be associated with the vegetative sterols which are from the constituents of *Echinacea* extract (Skaudickas et al., 2003; 2004). The antiandrogenic effect of the preparations of *E. purpurea* makes it possible to claim that it is associated with vegetative sterols (sitosterol, campesterol, stigmasterol) (Skaudickas et al., 2004). The chemical structure of these compounds is very similar to cholesterol. In the digestive duct, vegetative sterols reduce the absorption of cholesterol, creating a certain competition between sterols and cholesterols; thus, dietary phytosterols may lower the activities of the enzymes of testosterone metabolism (Awad and Fink, 2000; Trautwein and Demonty, 2007). However, some studies found different results, in which the testosterone level was increased in the administration of *E. pupurea* ethanol (EPE) compared to the control *in vivo* study (Mao et al., 2021). The explanation is that testosterone synthesis is catalyzed by an enzyme called 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3). This catalyzes the reduction of androstenedione to testosterone (Mindnich et al., 2005). The results of that study indicated that EPE extract, especially with a

dose of 465 mg/kg, was able to ameliorate the mRNA expression of kisspeptin receptor (Kiss1R), steroidogenic acute regulatory protein (StAR), and 17β-HSD3 (Mao et al., 2021). Kiss1R is the receptor of a neuropeptide called kisspeptin, which is associated with initiating puberty and maintaining the reproductive capacity of the adult (Wahab et al., 2018). Furthermore, StAR is a labile phosphoprotein known for the regulation of steroid hormones. The activity of StAR is highly enhanced in the adrenals and gonads under the acute steroidogenesis process (Manna et al., 2009).

Lastly, this study shows that the administration of *E. purpurea* can up-regulate the expression of estrogen hormone in females. The possible mechanism remains unclear, while another study shows that *Echinacea* has not shown estrogenic activity (Zava et al., 1998). However, another study with a molecular docking approach to identify potential estrogen mimics or anti-estrogens in phytochemicals revealed six strongly docking compounds in *E. purpurea*: flavonoid quercetin; phenolic compounds caffeoyl-p-coumaroyltartaric acid, caftaric acid, and chicoric acid; and sesquiterpenoids cinnamoylechinadiol and cinnamoylepoxyechinadiol (Powers and Setzer, 2015). Besides that, there are also several possible pathways of androgen and estrogen in regulating TMPRSS2 expression. Androgens *in vivo* regulate TMPRSS2 expression, and TMPRSS2 is an androgen-responsive gene (Lucas et al., 2014; Qiao et al., 2021). TMPRSS2 is strongly up-regulated in prostate cancer cells in response to androgens (Lucas et al., 2014). Other studies also show that TMPRSS2 is not an estrogen-responsive gene, but estrogen can regulate TMPRSS2 expression (Treppiedi et al., 2022). Lastly, TMPRSS2 is an androgen-regulated gene and regulation of the oncogenic transmembrane protease, serine 2, and E26 transformation-specific related gene (TMPRSS2-ERG) fusion involves ER signaling pathways (Bonkhoff, 2018).

All of the aforementioned mechanisms explain that *E. purpurea* has an effect on the regulation of reproductive hormones, which indirectly adds to strong evidence of its function as an immunomodulator. In addition, the effect on the expression of TMPRSS2, both through the reduction of pro-inflammatory cytokines and its indirect effect through the regulatory pathways for the two reproductive hormones above, provides additional evidence of the potential effect of *E. purpurea* as an antiviral and protection against SARS-COV2 by several mechanisms caused by down-regulation of TMPRSS2 itself including reduced viral entry (Leach et al., 2021; Mahmoud and Jarrar, 2021), altered site infection (Leach et al., 2021; Liu et al., 2022), and inhibition of viral replication (Wettstein et al., 2022).

Limitation of study

It is important to acknowledge the limitations inherent in this study. Firstly, the study was conducted with a relatively small sample size. A larger sample size would provide more robust and generalizable results, reducing the potential for sampling bias and increasing the statistical power of the study. Secondly, the study participants were selected based on specific inclusion and exclusion criteria, which may limit the generalizability of the findings to broader populations. Thirdly, the duration may have been relatively short. Lastly, although potential molecular mechanisms were discussed, the exact mechanisms through which *E. purpurea* parts may affect reproductive hormones require further investigation. The complex interactions between the bioactive compounds in *E. purpurea* and hormonal pathways necessitate more in-depth studies to elucidate the precise mechanisms involved.

CONCLUSION

The results of this study demonstrated several notable findings, including that *E. purpurea* may be capable of down-regulating androgen in males and up-regulating estrogen in females, in addition to down-regulating TMPRSS2 expression. Despite these encouraging results, several limitations should be considered. Further research with larger participant numbers may improve the generalizability of the results, and longer follow-up periods are warranted to validate and expand upon these findings. Future studies should also consider incorporating additional outcome measures. Lastly, this study contributes to the growing body of literature exploring the effects of *E. purpurea* as an antiviral property, especially for SARS-CoV-2, and its effect on reproductive hormones.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Agrawal AD (2011) Pharmacological activities of flavonoids: A review. Int J Pharm Sci Nanotechnol 4(2): 1394–1398. https://doi.org/10.37285/ijpsn.2011.4.2.3

Attarzadeh M, Balouchi H, Rajaie M, Dehnavi MM, Salehi A (2020) Improving growth and phenolic compounds of *Echinacea purpurea* root by integrating biological and chemical resources of phosphorus under water deficit stress. Ind Crops Prod 154: 112763. https://doi.org/10.1016/j.indcrop.2020.112763

Attarzadeh M, Balouchi H, Rajaie M, Movahhedi Dehnavi M, Salehi A (2019) Growth and nutrient content of *Echinacea purpurea* as affected by the combination of phosphorus with arbuscular mycorrhizal fungus and *Pseudomonas florescent* bacterium under different irrigation regimes. J Environ Manage 231: 182–188. https://doi.org/10.1016/j.jenvman.2018.10.040

Awad AB, Fink CS (2000) Phytosterols as anticancer dietary components: Evidence and mechanism of action. J Nutr 130(9): 2127–2130. https://doi.org/10.1093/jn/130.9.2127

Balciunaite G, Juodsnukyte J, Savickas A, Ragazinskiene O, Siatkute L, Zvirblyte G, Mistiniene E, Savickiene N (2015) Fractionation and evaluation of proteins in roots of *Echinacea purpurea* (L.) Moench. Acta Pharm 65(4): 473–479. https://doi.org/10.1515/acph-2015-0036

Bergeron C, Gafner S (2007) Quantitative analysis of the polysaccharide and glycoprotein fractions in *Echinacea purpurea* and *Echinacea angustifolia* by HPLC-ELSD for quality control of raw material. Pharm Biol 45(2): 98–105. https://doi.org/10.1080/13880200601112893

Bodinet C, Beuscher N (1991) Antiviral and immunological activity of glycoproteins from *Echinacea purpurea* radix. Planta Med 57(S 2): A33–A34. https://doi.org/10.1055/s-2006-960283

Bonkhoff H (2018) Estrogen receptor signaling in prostate cancer: Implications for carcinogenesis and tumor progression. Prostate 78(1): 2–10. https://doi.org/10.1002/pros.23446

Bruni R, Brighenti V, Caesar LK, Bertelli D, Cech NB, Pellati F (2018) Analytical methods for the study of bioactive compounds from medicinally used *Echinacea* species. J Pharm Biomed Anal 160: 443–477. https://doi.org/10.1016/j.jpba.2018.07.044

Cai C, Chen Y, Zhong S, Ji B, Wang J, Bai X, Shi G (2014) Anti-Inflammatory activity of n-butanol extract from *Ipomoea stolonifera in vivo* and *in vitro*. PLoS One 9(4): e95931. https://doi.org/10.1371/journal.pone.0095931

Cech NB, Kandhi V, Davis JM, Hamilton A, Eads D, Laster SM (2010) Echinacea and its alkylamides: Effects on the influenza A-induced secretion of cytokines, chemokines, and PGE2 from RAW 264.7 macrophage-like cells. Int Immunopharmacol 10(10): 1268–1278. https://doi.org/10.1016/j.intimp.2010.07.009

Cioccarelli C, Sánchez-Rodríguez R, Angioni R, Venegas FC, Bertoldi N, Munari F, Cattelan A, Molon B, Viola A (2021) IL1β Promotes TMPRSS2 expression and SARS-CoV-2 cell entry through the p38 MAPK-GATA2 axis. Front Immunol 12: 781352. https://doi.org/10.3389/fimmu.2021.781352

Dalby-Brown L, Barsett H, Landbo A-KR, Meyer AS, Mølgaard P (2005) Synergistic antioxidative effects of alkamides, caffeic acid derivatives, and polysaccharide fractions from *Echinacea purpurea* on *in vitro* oxidation of human low-density lipoproteins. J Agric Food Chem 53(24): 9413–9423. https://doi.org/10.1021/jf0502395

Del-Rio-Navarro BE, Espinosa-Rosales FJ, Flenady V, Sienra-Monge JJ (2006) Immunostimulants for preventing respiratory tract infection in children. Cochrane Database of Syst Rev https://doi.org/10.1002/14651858.CD004974.pub2

El-Ashmawy NE, El-Zamarany EA, Salem ML, El-Bahrawy HA, Al-Ashmawy GM (2015) *In vitro* and *in vivo* studies of the immunomodulatory effect of *Echinacea purpurea* on dendritic cells. J Genet Eng Biotechnol 13(2): 185–192. https://doi.org/10.1016/j.jgeb.2015.05.002

Fast DJ, Balles JA, Scholten JD, Mulder T, Rana J (2015) *Echinacea purpurea* root extract inhibits TNF release in response to Pam3Csk4 in a phosphatidylinositol-3-kinase dependent manner. Cell Immunol 297(2): 94–99. https://doi.org/10.1016/j.cellimm.2015.07.003

Gubernatorova EO, Gorshkova EA, Polinova AI, Drutskaya MS (2020) IL-6: Relevance for immunopathology of SARS-CoV-2. Cytokine Growth Factor Rev 53: 13–24. https://doi.org/10.1016/j.cytogfr.2020.05.009

Guiotto P, Woelkart K, Grabnar I, Voinovich D, Perissutti B, Invernizzi S, Granzotto M, Bauer R (2008) Pharmacokinetics and immunomodulatory effects of phytotherapeutic lozenges (bonbons) with *Echinacea purpurea* extract. Phytomedicine 15(8): 547–554. https://doi.org/10.1016/j.phymed.2008.05.003

Haria EN, Perera MADN, Senchina DS (2016) Immunomodulatory effects of *Echinacea laevigata* ethanol tinctures produced from different organs. Biosci Horiz 9: hzw001. https://doi.org/10.1093/biohorizons/hzw001

Indalao IL, Sawabuchi T, Takahashi E, Kido H (2017) IL-1β is a key cytokine that induces trypsin upregulation in the influenza virus–cytokine–trypsin cycle. Arch Virol 162(1): 201–211. https://doi.org/10.1007/s00705-016-3093-3

Iwata-Yoshikawa N, Kakizaki M, Shiwa-Sudo N, Okura T, Tahara M, Fukushi S, Maeda K, Kawase M, Asanuma H, Tomita Y, Takayama I, Matsuyama S, Shirato K, Suzuki T, Nagata N, Takeda M (2022) Essential role of TMPRSS2 in SARS-CoV-2 infection in murine airways. Nat Commun 13(1): 6100. https://doi.org/10.1038/s41467-022-33911-8

Jiang W, Zhu H, Xu W, Liu C, Hu B, Guo Y, Cheng Y, Qian H (2021) *Echinacea purpurea* polysaccharide prepared by fractional precipitation prevents alcoholic liver injury in mice by protecting the intestinal barrier and regulating liver-related pathways. Int J Biol Macromol 187: 143–156. https://doi.org/10.1016/j.ijbiomac.2021.07.095

Kakimov A, Muratbayev A, Zharykbasova K, Amanzholov S, Mirasheva G, Kassymov S, Utegenova A, Jumazhanova M, Shariati MA (2021) Heavy metals analysis, GCMS-QP quantification of flavonoids, amino acids and saponins, analysis of tannins and organoleptic properties of powder and tincture of *Echinacea purpurea* (L.) and *Rhapónticum carthamoídes*. Potr S J F Sci 15: 330–339. https://doi.org/10.5219/1476

Kim H-R, Oh S-K, Lim W, Lee HK, Moon B-I, Seoh J-Y (2014) Immune enhancing effects of *Echinacea purpurea* root extract by reducing regulatory t cell number and function. Nat Prod Commun 9(4): 511–514. https://doi.org/10.1177/1934578X1400900422

Kumar KM, Ramaiah S (2011) Pharmacological importance of *Echinacea purpurea*. Int J Pharma Biosci 2(4): 304–314.

Kurkin VA, Akushskaya AS, Avdeeva EV, Velmyaikina EI, Daeva ED, Kadentsev VI (2011) Flavonoids from *Echinacea purpurea*. Russ J Bioorg Chem 37(7): 905–906. https://doi.org/10.1134/S1068162011070120

Leach DA, Mohr A, Giotis ES, Cil E, Isac AM, Yates LL, Barclay WS, Zwacka RM, Bevan CL, Brooke GN (2021) The antiandrogen enzalutamide downregulates TMPRSS2 and reduces cellular entry of SARS-CoV-2 in human lung cells. Nat Commun 12(1): 4068. https://doi.org/10.1038/s41467-021-24342-y

Lemeshow S, David WH (1997) Large Samples in Health Research. Yogyakarta: Gadjah Mada University Press.

Li Y, Wang Y, Wu Y, Wang B, Chen X, Xu Xin, Chen H, Li W, Xu X (2017) *Echinacea pupurea* extracts promote murine dendritic cell maturation by activation of JNK, p38 MAPK and NF-κB pathways. Dev Comp Immunol 73: 21–26. https://doi.org/10.1016/j.dci.2017.03.002

Liu X, Liu B, Shang Y, Cao P, Hou J, Chen F, Zhang B, Fan Y, Tan K (2022) Decreased TMPRSS2 expression by SARS-CoV-2 predicts the poor prognosis of lung cancer patients through metabolic pathways and immune infiltration. Aging 14(1): 73–108. https://doi.org/10.18632/aging.203823

Lucas JM, Heinlein C, Kim T, Hernandez SA, Malik MS, True LD, Morrissey C, Corey E, Montgomery B, Mostaghel E, Clegg N, Coleman I, Brown CM, Schneider EL, Craik C, Simon JA, Bedalov A, Nelson PS (2014) The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. Cancer Discov 4(11): 1310–1325. https://doi.org/10.1158/2159-8290.CD-13-1010

Maggini V, De Leo M, Granchi C, Tuccinardi T, Mengoni A, Gallo ER, Biffi S, Fani R, Pistelli L, Firenzuoli F, Bogani P (2019) The influence of *Echinacea purpurea* leaf microbiota on chicoric acid level. Sci Rep 9(1): 10897. https://doi.org/10.1038/s41598-019-47329-8

Mahapatra S, Rattan R, Mohanty CBK (2021) Convalescent plasma therapy in the management of COVID-19 patients-The newer dimensions. Transfus Clin Biol 28(3): 246–253. https://doi.org/10.1016/j.tracli.2021.04.009

Mahmoud IS, Jarrar YB (2021) Targeting the intestinal TMPRSS2 protease to prevent SARS-CoV-2 entry into enterocytes-prospects and challenges. Mol Biol Rep 48(5): 4667–4675. https://doi.org/10.1007/s11033-021-06390-1

Manna PR, Dyson MT, Stocco DM (2009) Regulation of the steroidogenic acute regulatory protein gene expression: Present and future perspectives. Mol Hum Reprod 15(6): 321–333. https://doi.org/10.1093/molehr/gap025

Mao C-F, Sudirman S, Lee C-C, Tsou D, Kong Z-L (2021) *Echinacea purpurea* ethanol extract improves male reproductive dysfunction with streptozotocin–nicotinamide-induced diabetic rats. Front Vet Sci 8: 651286. https://doi.org/10.3389/fvets.2021.651286

Mindnich R, Haller F, Halbach F, Moeller G, de Angelis MH, Adamski J (2005) Androgen metabolism via 17β-hydroxysteroid dehydrogenase type 3 in mammalian and non-mammalian vertebrates: Comparison of the human and the zebrafish enzyme. J Mol Endocrinol 35(2): 305–316. https://doi.org/10.1677/jme.1.01853

Mohamed Sharif KO, Tufekci EF, Ustaoglu B, Altunoglu YC, Zengin G, Llorent-Martínez EJ, Guney K, Baloglu MC (2021) Anticancer and biological properties of leaf and flower extracts of *Echinacea purpurea* (L.) Moench. Food Biosci 41: 101005 https://doi.org/10.1016/j.fbio.2021.101005

Mudge E, Lopes-Lutz D, Brown P, Schieber A (2011) Analysis of alkylamides in Echinacea plant materials and dietary supplements by ultrafast liquid chromatography with diode array and mass spectrometric detection. J Agric Food Chem 59(15): 8086–8094. https://doi.org/10.1021/jf201158k

Nagoor Meeran MF, Javed H, Sharma C, Goyal SN, Kumar S, Jha NK, Ojha S (2021) Can *Echinacea* be a potential candidate to target immunity, inflammation, and infection - The trinity of coronavirus disease 2019. Heliyon 7(2): e05990. https://doi.org/10.1016/j.heliyon.2021.e05990

Novika RGH, Wahidah NJ, Rahmawati NY, Ansori ANM (2022) *Apium graveolens* and *Eucalyptus globulus* decrease stress and protect folliculogenesis marker on woman reproductive health during COVID-19 pandemic. Indones J Pharm 33: 592–601. https://doi.org/10.22146/ijp.4511

Nyalambisa M, Oyemitan IA, Matewu R, Oyedeji OO, Oluwafemi OS, Songca SP, Nkeh-Chungag BN, Oyedeji AO (2017) Volatile constituents and biological activities of the leaf and root of *Echinacea* species from South Africa. Saudi Pharm J 25(3): 381–386. https://doi.org/10.1016/j.jsps.2016.09.010

Pallag A, Bungau S, Tit DM, Jurca T, Sirbu V, Honiges A, Horhogea C (2016) Comparative study of polyphenols, flavonoids and chlorophylls in *Equisetum arvense* L. populations. Rev Chim 67: 530–533.

Powers CN, Setzer WN (2015) A molecular docking study of phytochemical estrogen mimics from dietary herbal supplements. In Silico Pharmacol 3(1): 4. https://doi.org/10.1186/s40203-015-0008-z

Qiao Y, Wang X-M, Mannan R, Pitchiaya S, Zhang Y, Wotring JW, Xiao L, Robinson DR, Wu Y-M, Tien JC-Y, Cao X, Simko SA, Apel IJ, Bawa P, Kregel S, Narayanan SP, Raskind G, Ellison SJ, Parolia A, Zelenka-Wang S, McMurry L, Su F, Wang R, Cheng Y, Delekta AD, Mei Z, Pretto CD, Wang S, Mehra R, Sexton JZ, Chinnaiyan AM (2021) Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TMPRSS2. Proc Natl Acad Sci USA 118(1): e2021450118. https://doi.org/10.1073/pnas.2021450118

Ramezannezhad R, Aghdasi M, Fatemi M (2019) Enhanced production of cichoric acid in cell suspension culture of *Echinacea purpurea* by silver nanoparticle elicitation. Plant Cell Tiss Organ Cult 139(2): 261–273. https://doi.org/10.1007/s11240-019-01678-4

Renteria AE, Mfuna Endam L, Adam D, Filali-Mouhim A, Maniakas A, Rousseau S, Brochiero E, Gallo S, Desrosiers M (2020) Azithromycin downregulates gene expression of IL-1β and pathways involving TMPRSS2 and TMPRSS11D required by SARS-CoV-2. Am J Respir Cell Mol Biol 63(5): 707–709. https://doi.org/10.1165/rcmb.2020-0285LE

Rios MY, Olivo HF (2014) Natural and synthetic alkamides: Applications in pain therapy. Studies in Natural Products Chemistry 43: 79-121. https://doi.org/10.1016/B978-0-444-63430-6.00003-5

Roesler J, Emmendörffer A, Steinmüller C, Luettig B, Wagner H, Lohmann-Matthes M-L (1991) Application of purified polysaccharides from cell cultures of the plant *Echinacea purpurea* to test subjects mediates activation of the phagocyte system. Int J Immunopharmacol 13(7): 931–941. https://doi.org/10.1016/0192-0561(91)90046-A

Saeidnia S, Manayi A, Vazirian M (2015) *Echinacea purpurea*: Pharmacology, phytochemistry and analysis methods. Pharmacogn Rev 9(17): 63. https://doi.org/10.4103/0973-7847.156353

Skaudickas D, Kondrotas A, Baltrusaitis K (2004) The effect of *Echinacea purpurea* extract on sexual glands of male rats. Medicina (Kaunas, Lithuania) 40(12): 1211–1218.

Skaudickas D, Kondrotas AJ, Baltrusaitis K, Vaitiekaitis G (2003) Effect of Echinacea (*Echinacea purpurea* L. Moench) preparations on experimental prostate gland. Medicina (Kaunas, Lithuania) 39(8): 761–766.

Tabar RS, Moieni A, Monfared SR (2019) Improving biomass and chicoric acid content in hairy roots of *Echinacea purpurea* L. Biologia 74(8): 941–951. https://doi.org/10.2478/s11756-019-00232-z

Trautwein EA, Demonty I (2007) Phytosterols: natural compounds with established and emerging health benefits. OCL 14(5): 259–266. https://doi.org/10.1051/ocl.2007.0145

Treppiedi D, Marra G, Di Muro G, Catalano R, Mangili F, Esposito E, Barbieri AM, Arosio M, Mantovani G, Peverelli E (2022) TMPRSS2 Expression and activity modulation by sex-related hormones in lung Calu-3 cells: Impact on gender-specific SARS-CoV-2 infection. Front Endocrinol 13: 862789. https://doi.org/10.3389/fendo.2022.862789

Vendramin V, Viel A, Vincenzi S (2021) Caftaric acid isolation from unripe grape: A "green" alternative for hydroxycinnamic acids recovery. Molecules 26(4): 1148. https://doi.org/10.3390/molecules26041148

Vieira SF, Gonçalves VMF, Llaguno CP, Macías F, Tiritan ME, Reis RL, Ferreira H, Neves NM (2022) On the bioactivity of *Echinacea purpurea* extracts to modulate the production of inflammatory mediators. Int J Mol Sci 23(21): 13616. https://doi.org/10.3390/ijms232113616

Vimalanathan S, Shehata M, Sadasivam K, Delbue S, Dolci M, Pariani E, D'Alessandro S, Pleschka S (2022) Broad antiviral effects of *Echinacea purpurea* against SARS-CoV-2 variants of concern and potential mechanism of action. Microorganisms 10(11): 2145. https://doi.org/10.3390/microorganisms10112145

Wahab F, Atika B, Ullah F, Shahab M, Behr R (2018) Metabolic impact on the hypothalamic kisspeptin-Kiss1r signaling pathway. Front Endocrinol 9: 123. https://doi.org/10.3389/fendo.2018.00123

Wettstein L, Kirchhoff F, Münch J (2022) The transmembrane protease TMPRSS2 as a therapeutic target for COVID-19 treatment. Int J Mol Sci 23(3): 1351. https://doi.org/10.3390/ijms23031351

World Health Organization (1999) WHO monographs on selected medicinal plants. Geneva: World Health Organization.

World Medical Association (2013) World Medical Association Declaration of Helsinki. JAMA 310(20): 2191. https://doi.org/10.1001/jama.2013.281053

Xu W, Hu B, Cheng Y, Guo Y, Yao W, Qian H (2022) *Echinacea purpurea* suppresses the cell survival and metastasis of hepatocellular carcinoma through regulating the PI3K/Akt pathway. Int J Biochem Cell Biol 142: 106–115. https://doi.org/10.1016/j.biocel.2021.106115

Yang G, Li K, Liu C, Peng P, Bai M, Sun J, Li Q, Yang Z, Yang Y, Wu H (2018) A comparison of the immunostimulatory effects of polysaccharides from tetraploid and diploid *Echinacea purpurea*. BioMed Res Int 2018: 8628531 . https://doi.org/10.1155/2018/8628531

Yao L, Bai L, Tan Y, Sun J, Qu Q, Shi D, Guo S, Liu C (2019) The immunoregulatory effect of sulfated *Echinacea purpurea* polysaccharide on chicken bone marrow-derived dendritic cells. Int J Biol Macromol 139: 1123–1132. https://doi.org/10.1016/j.ijbiomac.2019.08.028

Zava DT, Dollbaum CM, Blen M (1998) Estrogen and progestin bioactivity of foods, herbs, and spices. Exp Biol Med 217(3): 369–378. https://doi.org/10.3181/00379727-217-44247

Zhai Z, Liu Y, Wu L, Senchina DS, Wurtele ES, Murphy PA, Kohut ML, Cunnick JE (2007) Enhancement of innate and adaptive immune functions by multiple *Echinacea* species. J Med Food 10(3): 423–434. https://doi.org/10.1089/jmf.2006.257

AUTHOR CONTRIBUTION:

Contribution	Novika RGH	Wahidah NJ	Yunus A	Sumarno L	Ilyas MF
Concepts or ideas	x			x	
Design	x			x	
Definition of intellectual content	x		x	x	x
Literature search	x		x		x
Clinical trial	x	x			
Experimental studies	x				
Data acquisition	x			x	
Data analysis	x				
Statistical analysis	x				
Manuscript preparation		x	x		x
Manuscript editing	x		x		x
Manuscript review	x	x	x	x	x

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