

In vivo Evaluation of Antifertility Activity of Aqueous and Butanol Fractions of Methanolic Root Extract of *Rumex steudelii* in Female Mice and Rats

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Abstract

Background: In Ethiopia, to control fertility, traditional medicines have been used for many years. The crude methanolic root extract of *Rumex steudelii* (Tult) is believed to have anti-fertility effect.

Objectives: To identify fraction(s) of crude methanolic root extract of *Rumex steudelii* responsible for antifertility effect, to suggest the possible mechanisms, to determine the oral Lethal Dose, 50% (LD₅₀), and to screen the phytochemicals for the fractions.

Methods: Preliminary screening for the antifertility activity was conducted in female mice at 700mg/kg and 900mg/kg using aqueous and butanol fraction from January to November, 2011, Addis Ababa, Ethiopia. Besides effect of the fractions at 900mg/kg on implantation, serum estrogen, progesterone, and cholesterol, the weight of genital organ and body weight was carried out.

Results: The fractions reduced the number of litters at 700mg/kg and 900mg/kg ($p < 0.01$). The aqueous fraction decreased implantation site ($p < 0.05$) which was further supported by increase in serum progesterone level ($p < 0.001$). The wet weight of the uterus was shown also to be reduced significantly ($p < 0.05$). The LD₅₀ of the aqueous and butanol fractions was found to be 10.475g/kg and 7.080g/kg, respectively. Determination of the secondary metabolites in these fractions indicated the presence of phytosterols, polyphenols and tannins, which could be responsible constituents for their antifertility effects.

Conclusion: The aqueous fraction showed more antifertility activity and higher lethal dose than butanol fraction. Further investigations on the quantity of screened phytochemicals and efficacy, safety, isolation, characterization and structural elucidation of active principles on these fractions should be conducted. Moreover the antifertility effects and phytochemical screening of other fractions need to be investigated.

Keywords: *Rumex steudelii*, antifertility, aqueous fraction, butanol fraction, LD₅₀, secondary metabolite

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Introduction

The ever increasing population in the world, particularly in low income countries, has detrimental effects on life supporting system on earth. Fertility regulation comprising contraception and management of infertility forms an important component of reproductive health (Gupta and Sharma, 2006). There are different methods used for family planning methods: continence abstinence, rhythm method, barriers, hormonal contraceptives, implantable devices, and permanent birth control methods (Qureshi *et al.*, 2006). But there is no "best" method of birth control. Each method has its pros and cons making choices difficult (USAID, 2010).

The development of new fertility regulating drugs from medicinal plants is an attractive proposition for a number of reasons. Plants derived compounds or their derivatives form the basis of a large number of established drugs. Approximately 80% of the world's populations depend on health care systems that involve the use of traditional medicine including fertility regulating plants (WHO, 2001). The acceptability of new antifertility drugs may be greatly enhanced if these preparations are based on indigenous knowledge and practices (Bekele, 2007). Major economic and commercial benefits could be derived by fostering this

national self-reliance in drug development and production.

Natural plant substances that have mild inherent estrogenic and anti-estrogenic properties offer themselves as effective non-conventional sources of contraception with less deleterious side effects. For this reason, continuous efforts are being made to use natural plants to develop antifertility products (Angela and Christy, 2009).

The practice of traditional medicine for the control of fertility in rural Ethiopia is also based on folk use of numerous anti-fertility herbs. A total of 210 extracts/fractions from 70 traditionally used Ethiopian plants were subjected to utero-tonic and anti-implantation bioassays (Desta, 1994). *Achyranthes aspera* (Shibeshi *et al.*, 2006), *Asparagus africanus* (Tafesse *et al.*, 2006), *Leonotis ocyimifolia* (Tafesse *et al.*, 2005), *Jatropha curcas* L. (Makonnen *et al.*, 1997) and *Rumex steudelii* (Gebrie *et al.*, 2005b) are some of those used for this purpose.

Rumex steudelii, H. whose vernacular name is “Tult” or “Yeberemelase”, is found in different parts of Ethiopia, such as Tigray, Gondar, Gojam, Wollo, Shewa and Arsi highlands. It is an erect, perennial herb which grows up to one meter tall at an altitudinal range of 1200–3900m. It is traditionally used with other medicinal plants to treat various illnesses such as hemorrhoids, rectal prolapse, abdominal colic wounds, eczema, leprosy and tonsillitis. It is also used as hemostatic and oxytocic agent (Gebrie *et al.*, 2005a).

The methanolic extract of this plant showed anti-implantation effect in rats (Desta, 1994). It prolonged the estrous cycle and the diestrous phase of the estrous cycle (Gebrie *et al.*, 2005a). It also showed that the antifertility effects of the extract were transient and reversible. The extract caused significant decrease in the number of implantation sites and reduced the serum estrogen level (Gebrie *et al.*, 2005b). The phytochemical screening of the plant has showed the presence of polyphenols, phytosterols, saponins and tannins (Gebrie *et al.*, 2005a). Atrophic changes in the uterus and disruption of ovarian folliculogenesis by inhibiting further development of recruited ovarian follicles in dose dependent manner was also discovered (Tibebu *et al.*, 2010).

Despite the availability of several findings stating its antifertility effect, it was conducted using crude extracts (Gebrie *et al.*, 2005a; Gebrie *et al.*, 2005b; Tibebu *et al.*, 2010). Therefore, the objectives of the present study were to identify fraction(s) of crude methanolic root extract of *Rumex steudelii* responsible for antifertility

effect, to suggest the possible mechanisms, to determine the oral LD₅₀ (Lethal Dose in 50% of mice), and to screen phytochemical for the fractions in Addis Ababa, Ethiopia, from January to November, 2011.

Materials and Methods

Preparation of Animals

All anti-fertility experiments were performed on inbred adult, cyclic virgin female albino rats (4-5 months old and weighing 150-200g) except in preliminary screening where female albino mice (2-3 months old and weighing 25-30g) were used. Female albino mice weighing 25-30g were also used for the acute toxicity study. The mice used in these experiments were obtained from Ethiopian Public Health Institute. The animals were housed in polypropylene cages and maintained under environmentally controlled room provided with 12:12 hour light and dark cycle for each 24 hour period at a temperature of 25°C. They were fed on pellets and tap water ad libitum. The animals were allowed to acclimatize to the laboratory environment for three to five days prior to the experiment (Obernier and Baldwin, 2006).

Collection and Identification of Plant Material

The root of *Rumex steudelii* was collected from a garden in Ethiopian Public Health Institute by a staff member in December, 2010. The plant was identified by a taxonomist and a voucher sample (Herbarium number AA-2135) was deposited in the herbarium of the Department of Drug Research of Ethiopian Public Health Institute, Addis Ababa.

Preparation of Plant Crude Extract

The crude extract of *Rumex steudelii* was prepared based on the manual described by Debella (2002). The root of the plant was dried under shade, ground into coarse powder, and percolated in 80% methanol for three days. Then it was filtered using filter paper (Whatman No. 1). The solvent was removed by using rotary evaporator. Further concentration of the extract was made by heating and the evaporation of the solvent was kept in water bath at 40°C, which finally gave a brownish dark semi-solid mass. The percent yield of 1,103g powdered *Rumex steudelii* root was 23.4%w/w (258.1g) methanolic extract.

Preparation of Solvent Fractions

The isolation of compounds from natural sources in a pure state is very important, but it can be a difficult and time-consuming process in natural product research. One of the separation techniques is the solvent partitioning method, which usually involves the use of two immiscible solvents in a separating funnel. In this method, compounds were distributed in two solvents according to their different partition coefficients. This technique is highly effective, as the first step of the fairly large-scale separation of compounds from crude natural product extracts (Otsuka, 2005).

The crude *Rumex steudelii* root extract was fractionated by dissolving 50g methanolic root extract in mild hot distilled water. After filtration, the dissolved extract was added in a separating funnel and mixed with 50ml of n-hexane three times. After shaking, it was allowed to stay for some time until a complete formation of two layers. Then the upper layer, i.e. n-hexane, was collected. After collecting the n-hexane layer, 50ml of dichloromethane was successively added three times in the left aqueous residue and then the lower layer (dichloromethane) was taken. After 50ml of n-butanol was added successively three times in the remaining aqueous residue, the upper layer, i.e. n-butanol, was collected. Finally, n-hexane, dichloromethane and n-butanol fractions were concentrated under vacuum using rotary evaporator and then dried at 40°C using water bath to give 0.029%(w/w), 0.035%(w/w), 31.7%(w/w) residue, respectively. But the aqueous fraction residue was lyophilized (freeze-dried) to give 32.5% (w/w). Since the yield for hexane and dichloromethane were too little, n-butanol and aqueous fractions were used in the experiments. The n-butanol fraction was stored in refrigerator at -8°C and aqueous fraction in desiccator until the experiment was conducted.

Test Material Administration and Dose Calculation

The administration of the extract was done with intragastric tube on the basis of the animal's body weight. The dose for each animal was calculated, considering the doses that were effective in the previous studies conducted by Gebrie *et al.* (2005a) on methanol extract of *Rumex steudelii* root. Based on the percent of yield of the crude extract for the fractions, the proportion of these doses which could give the dose for the fractions was calculated, as was done in other study (Flores *et al.*, 2008). Hence two doses were selected: 2.2g/kg and 2.8g/kg. The proportions of aqueous fraction were 0.715g and 0.910g, and butanol fractions

were also found to be 0.697g and 0.888g, respectively. Moreover, from acute toxicity study, the result of LD₅₀ of crude extract and pilot study were also considered based on Organization of Economic Co-operation and Development guideline (OECD, 2008). To employ uniform doses, 0.7g/kg and 0.9g/kg were used. The aqueous and butanol fractions were reconstituted in distilled water and distilled water with 2% tween-80, respectively, to get the desired concentration for all pharmacological tests.

Pharmacological Screening for Antifertility Effect

Preliminary Screening for the Antifertility Activity

Six groups of mature female mice (6 mice per group) were selected for the experiment. Two groups were used as a negative control receiving distilled water and 2% tween-80 in distilled water vehicles for aqueous and butanol fractions, respectively. The remaining four groups received aqueous and butanol fractions at two doses levels each: 700mg/kg and 900mg/kg. All the groups received the test and control substances intragastrically daily for 7 days. All the experimental animals were then allowed to mate with mature male mice (one male for two female) and the administration of the vehicles and the fractions continued for 21 days. As described by Gebrie *et al.* (2005a), in both the control and test groups, the number of litters was determined after the completion of one gestation period. The litters of extract treated mice were then allowed to grow in order to check for postnatal growth and congenital anomalies.

Studies on Anti-implantation Activity

Four groups of mature female rats (6 rats per group) were selected for this experiment. Two groups served as negative control and received the vehicles intragastrically for 10 days. The other two groups were used as test groups receiving the aqueous and the butanol fractions at 900mg/kg for the same number of days by the same route. Anti-implantation activity was determined based on the methods used by Ahirwar *et al.* (2010) and Gebrie *et al.* (2005b). All the groups were left over night with male (in the ratio of 2 female to 1 male) and the vaginal smear was examined for motile spermatozoa in the next morning. The day on which the spermatozoa were found in the smear was considered the first day of pregnancy (day one). The animals were separated immediately after confirming mating. On the 11th day of pregnancy, all the groups of rats were undergone laparotomy under diethyl

ether anesthesia to determine the number of implantation sites in the horns of the uterus. The presence of significant difference in the mean number of implantation sites between the fractions and the controls were taken as a positive response.

Effect on Serum Estrogen, Progesterone and Cholesterol

In this experiment the two test groups (5 female rats/group) were treated with test substances at 900mg/kg for 10 days by intragastric gavages. The negative control groups were treated with vehicle in the same way as the test groups. The level of estrogen, progesterone, and cholesterol were determined based on the methods described by Gebrie *et al.* (2005b). On the 11th day, the control and the test groups were anesthetized under diethyl ether and blood was drawn by cardiac puncture. The blood was allowed to coagulate for an hour. The separation of the serum from other cellular components of the blood was done by centrifuging the coagulated blood at 2500 revolution/minute for 15 minutes. Then sera was collected and stored in deep freezer (-20 °C). After two weeks, the sera were analyzed for estrogen and progesterone using Elecsys2010 immunoassay analyzer. All components and reagents for routine analysis are integrated in or on the analyzer. Total cholesterol in serum was analyzed using the instrument Cobas Integra 400 Plus, Roche Diagnostics.

Effect of the Fractions on the Weight of Genital Organ and Body Weight

Four groups of matured female rats (6 rats per group) were selected for this experiment. Two groups served as negative control and received the vehicles intragastrically for 10 days. The other two groups were used as test group receiving the aqueous and the butanol fractions at 900mg/kg for the same number of days by the same route. On the 11th day, all the animals in all groups were weighed and sacrificed under diethyl ether anesthesia. The ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper, and weighed quickly on analytical balance. The ovary and the uterine ratios were calculated by dividing the ovary and the uterine weight in milligrams by body weight in grams (Gebrie *et al.*, 2005b).

Determination of Oral LD₅₀

The LD₅₀ of the fractions was determined by taking probit 5 (equivalent to 50% mortality) on the Y-axis

interpolated to X-axis to get log LD₅₀ and then antilogarithm of which was taken as the LD₅₀ of the fractions, as described by the Organization of Economic Co-operation and Development guideline (OECD, 2008). It was conducted in female Swiss albino mice. The fraction was administered by intragastric route starting from smaller to higher doses in 1ml of vehicle. The records of mortality and the manifestation of toxicity were made in 24 hours.

Secondary Metabolites Identification

Phytochemical tests of the aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* were carried out as described by Debella (2002). Phytosterols are screened by Lieberman and Burchard's reagent whereas polyphenols with 1% Ferric Chloride and 1% Potassium Ferrocyanide and formation of honeycomb froth was used to determine saponins. Moreover, 1% Potassium ferrocyanide and concentrated Ammonia, Sodium nitrate and 0.1N Hydrochloride acid, Ferric sulphate and Dragendroffs reagent were used to screen tannin, hydrolysable tannin, phenolic glycosides and alkaloids, respectively (Debella, 2002).

Statistical Analysis

The data were expressed as mean ± Standard Error of Mean (SEM) and analyzed using GraphPad Prism Version 5.04 software. One way Analysis of Variance (ANOVA) was used to assess the variation of the means among the treatments. Student paired t-test was performed to compare body weight changes before and after treatment. P-value less than 0.05 was considered as a cutoff point as statistically significant.

Ethical Considerations

This study was approved by the Ethics Review Committee of Addis Ababa University College of Health Sciences. Animal care and handling were according to international guidelines for the use and maintenance of experimental animals (Institute for Laboratory Animal Research, 1996; National Research Council, 2011; OECD, 2008).

Results

Preliminary Screening for the Antifertility Activity

Both fractions, aqueous and butanol, decreased the number of litters significantly ($p < 0.05$) at 700mg/kg.

Besides, there was no birth in those mice treated with 900mg/kg aqueous fraction and a significant ($p < 0.01$) decrease in number of litters was found in the mice that received butanol fraction at this dose level (Table 1).

Table 1. Antifertility effect of aqueous and butanol fractions of methanolic root extract *Rumex steudelii* at two dose levels on mice Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Number of animals	Mean number of litters (<i>Mean</i> \pm <i>SEM</i>)
Aqueous fraction	700	6	3 \pm 1.897*
	900	6	0.00
Negative control of aqueous fraction	Distilled water	6	10.333 \pm 1.542
Butanol fraction	700	6	4.123 \pm 2.0220*
	900	6	3.625 \pm 1.851**
Negative control of butanol fraction	2% tween 80 in distilled water	6	10.666 \pm 0.8819

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Anti-implantation Activity of Aqueous and Butanol Fractions of Methanolic Extract of Rumex steudelii

The aqueous fractions of methanolic root extract of *Rumex steudelii* showed a significant ($p < 0.05$) decrease in implantation site in the rats but the butanol fraction did not (Table 2).

Effect of Aqueous and Butanol Fractions of Methanolic Extract of Rumex steudelii on Serum Estrogen, Progesterone and Cholesterol

There was a significant ($p < 0.001$) increase in serum progesterone in the aqueous fraction treated rats but a decrease in serum cholesterol in fractions treated groups, as compared to the controls (Table 3).

Table 2. Effect of aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* on implantation, Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Number of animals	Number of implantation site (<i>Mean</i> \pm <i>SEM</i>)
Aqueous fraction	900	6	2.833 \pm 1.797*
Negative control of aqueous fraction	Distilled water	6	8.666 \pm 0.4216
Butanol fraction	900	6	4.833 \pm 1.537
Negative control of butanol fraction	2% tween-80 in distilled water	6	8.333 \pm 0.333

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3. Effect of aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* on serum estrogen, progesterone and cholesterol, Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Estrogen (pg/ml) (Mean ± SEM)	Progesterone (ng/ml) (Mean ± SEM)	Cholesterol (mg/dl) (Mean ± SEM)
Aqueous fraction	900	15.428 ± 1.861	49.768 ± 3.739***	52.2 ± 2.973
Negative control for aqueous fraction	Distilled water	14.654 ± 5.888	19.518 ± 5.37	60.6 ± 3.385
Butanol fraction	900	18.192 ± 3.268	15.634 ± 1.002	59 ± 8.602
Negative control for Butanol fraction	2% tween 80 in distilled water	19.742 ± 4.75	17.232 ± 7.312	72.4 ± 4.781

Note: Number of animals=5; pg: picogram; ng: nanogram; dl: deciliter; *p<0.05; **p<0.01; ***p<0.001

Effect of Aqueous and Butanol Fractions of Methanolic Extract of *Rumex steudelii* on the Weight of Genital Organ and Body Weight

Except in the butanol fraction group, all groups increased body weight significantly (p<0.001) after the study period, but a significant reduction in body weight

gain was observed in aqueous fraction (p<0.01) and butanol fraction (p<0.001) treated groups as compared to the controls. Uterine ratio was also significantly (p<0.05) reduced in the test groups compared to the controls. Ovarian ratio was not significantly affected with any of fractions (Table 4).

Table 4. Effect of aqueous and butanol fractions of methanolic root extract of *Rumex steudelii* on weight of genital organ and body weight, Addis Ababa, Ethiopia, 2011.

Treatment	Dose (mg/kg)	Uterine ratio (mg/gm) (Mean ± SEM)	Ovary ratio (mg/gm) (Mean ± SEM)	Body weight (gm) (Mean ± SEM)		Change in Body weight (gm) (Mean ± SEM)
				Initial	Final	
Aqueous fraction	900	1.1318 ± 0.1615*	0.5846 ± 0.0859 ^{NS}	154 ± 1.7703	171.9 ± 3.002***	17.7 ± 1.3**
Negative control of aqueous fraction	DW	1.7954 ± 0.1022	0.7906 ± 0.0699	153 ± 2.864	183.98 ± 1.195***	30.98 ± 2.94
Butanol fraction	900	1.3678 ± 0.0847*	0.6452 ± 0.0162	154 ± 1.461	163.233 ± 3.092	9.23 ± 1.804***
Negative control of Butanol fraction	2% tween 80 in DW	2.0462 ± 0.2262	0.8566 ± 0.083	151.4 ± 1.077	184.08 ± 1.951***	32.8 ± 0.9695

Note: Number of animals=6; *p<0.05; **p<0.01; ***p<0.001; DW: Distilled Water

LD₅₀ for Aqueous and Butanol Fractions of Methanolic Root Extract of *Rumex steudelii*

The LD₅₀ of aqueous (Figure 1) and butanol (Figure 2) fractions of methanolic root extract of *Rumex steudelii* were found to be 10.475g/kg and 7.08g/kg, respectively. During observation for toxic manifestations, most mice showed hypo-activity, piloerection, depression and breathing difficulty before death.

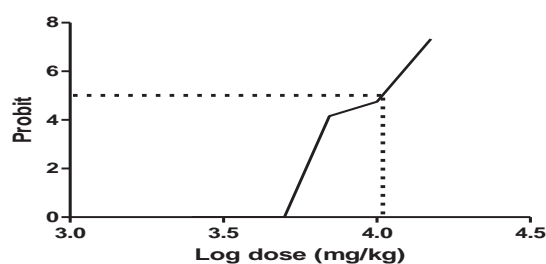


Figure 1. Lethal oral dose of aqueous fraction of methanolic root extract of *Rumex steudelii*, Addis Ababa, Ethiopia, 2011.

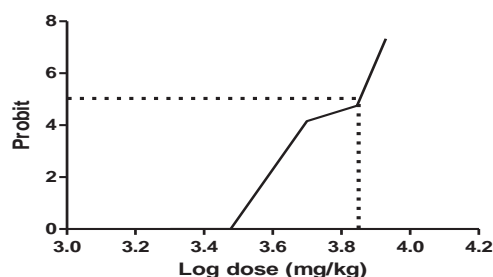


Figure 2. Lethal oral dose of butanol fraction of methanolic root extract of *Rumex steudelii*, Addis Ababa, Ethiopia, 2011.

Secondary Metabolites of the Aqueous and Butanol Fractions of Methanolic Root Extract of Rumex steudelii by Chemical Method

The aqueous fraction of methanolic root extract of *Rumex steudelii* was shown to have polyphenols, phytosterols, tannins and saponins (Table 5). Similar screening of butanol fraction displayed the presence of polyphenols, phytosteroids, tannins and hydrolysable tannins (Table 6).

Table 5. Secondary metabolites of aqueous fraction of methanolic root extract of *Rumex Steudelii* by chemical method, Addis Ababa, Ethiopia, 2011.

Plant material used	Secondary metabolites tested	Reagent	Result
Aqueous fraction	Polyphenols	1%K ₃ Fe(CN) ₆ & 1%FeCl ₃	+
	Phytosterols	Liebermann and Burchard's	+
	Alkaloids	Dragedroff's	-
	Tannins	1%K ₃ Fe(CN) ₆	+
	Hydrolysable tannins	NaNO ₃	-
	Saponins	Honeycomb froth formation	+
	Phenolic glycoside	FeSO ₄	-

Note: (+) indicate presence and (-) indicate absence of particular metabolites

Table 6. Secondary metabolites of butanol fraction of methanolic root extract of *Rumex Steudelii* by chemical method, Addis Ababa, Ethiopia, 2011.

Plant material used	Secondary metabolites tested	Reagent	Result
Butanol fraction	Polyphenols	1%K ₃ Fe(CN) ₆ & 1%FeCl ₃	+
	Phytosterols	Liebermann and Burchard's	+
	Alkaloids	Dragedroff's	-
	Tannins	1%K ₃ Fe(CN) ₆	+
	Hydrolysable tannins	NaNO ₃	+
	Saponins	Honeycomb froth formation	-
	Phenolic glycoside	FeSO ₄	-

Note: (+) indicate presence and (-) indicate absence of particular metabolites

Discussion

The antifertility activity of aqueous and butanol fractions of the 80% methanolic root extract of *Rumex steudelii* (crude extract) were conducted using different parameters. In preliminary screening of these fractions at two dose levels (700mg/kg and 900 mg/kg) on female mice for the treatment period of 28 days, the mean number of litters decreased very significantly with no delivered pups in those which received the aqueous fraction at 900mg/kg, indicating that these fractions

have antifertility effect. Anti-implantation activity and an increase in the level of serum progesterone were also observed with the aqueous fraction that could be possible mechanisms. The observations that both fractions had LD₅₀ greater than 5gm/kg clarify the safety nature of the fractions. The phytochemical screening of the fractions indicated the presence of phytosterols, polyphenols and tannins, which could possibly reason out the antifertility effect of the plant.

Based on these promising results in preliminary screening of the fractions, further experiments were conducted to establish the possible mechanisms for antifertility activity. Fractions treated mice's litters were allowed to grow to check for postnatal growth and congenital anomalies, but there was not any physical deformity observed in any of the litters. All litters grew into adult stage which suggests that the aqueous and butanol fractions of *Rumex stendelii* methanolic root extract may not have teratogenic effect even if contraception fails. Similar results were also seen with the methanolic crude extract of the plant (Gebrie *et al.*, 2005a).

Anti-implantation activity was observed with the aqueous fraction and this can be one possible mechanism for its antifertility effect. Similar results were reported on crude extract of this plant (Desta, 1994; Gebrie *et al.*, 2005b). Anti-implantation effects were also observed with *Trigonella foenum-graecum* (Ahirwar *et al.*, 2010), *Sida acuta* (Londonkar *et al.*, 2009), *Cassia fistula* (Yadav and Jain, 2009), *Ferula jaeschkeana* Vatke (Pathak and Prakash, 1989), and *Hibiscus rosa-sinensis* Linn (Vasudeva and Sharma, 2008).

The success of implantation depends on achieving the appropriate embryo development to the blastocyst stage, which in turn depends on the development of an endometrium. Implantation is a very intricate process, regulated by a number of complex molecules like hormones (estrogen and progesterone), cytokines (Leukemia inhibitory factor, Interleukin 6, Interleukin 11), and growth factors (Transforming growth factor- β , Epidermal growth factors, Heparin binding-epidermal growth factor, Insulin-like growth factors) and their cross talk. A network of these molecules plays a crucial role in preparing receptive endometrium and blastocyst (Singh *et al.*, 2011). Any imbalances or disturbances to these compounds could result in failure of implantation.

The increase in the level of serum progesterone with the aqueous fraction could explain its anti-implantation effect. Furthermore, insignificant anti-implantation effect of the butanol extract might be associated with its insignificant effect on the hormonal level. Its antifertility activity might be due to reduced uterine ratio and repeated administration.

Progesterone containing contraceptives act by inhibiting ovulation which result in low level of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), as well as the inadequacy of the mid cycle LH surge. Since the proper frequency of LH pulses is essential for ovulation, progesterone is likely to play a

major role in contraception. Progesterone also influences the endocervical glands, and the abundant watery secretion of the estrogen-stimulated structures is changed to a scant, viscid material thereby decreasing the penetration of the cervix by sperm, delaying the tubal transport of egg or embryo, and affecting fertilization. Besides, it is involved in the alteration of endometrial receptivity for implantation (Guyton and Hall, 2006).

Endometrium is known to become receptive only for short period in both rodents and humans. Beyond this period of receptivity, the embryo is unable to establish contact with receptive endometrium successfully. Therefore, a timely arrival of embryo in a receptive endometrium is very crucial for successful implantation (Singh *et al.*, 2011). The increased progesterone level by the aqueous fraction could, therefore, be possible mechanism for its anti-implant effect. A similar finding was reported by Akpantah *et al.* (2010) on Gonadal histomorphologies and serum hormonal milieu in female rats treated with *Azadirachta indica* leaf extract.

In the preliminary screening with the butanol fraction a significant reduction in the number of litters in the mice was shown, though the implantation sites in the rats were not affected. This could be attributed to the long term administration, which might cause fetal resorption. A similar result was seen on antifertility activity of *Deris brevipes* variety *coriacea* by Badami *et al.* (2003).

Estrogen increases body weight, uterine, and ovarian (Guyton and Hall, 2006). In this study, after treated with the aqueous and butanol fractions, there was a significant reduction in the body weight gain in both fractions as compared to the controls. Moreover, the reduced uterine and ovarian ratio which was observed with both fractions may indicate their anti-estrogenic nature which alters the biochemical milieu of the reproductive tract leading to change in the normal status of the reproduction in female reproductive tract of rat and thus may contribute for antifertility effect. Anti-estrogenic effects were also observed with *Nelumbo nucifera* (Mutreja *et al.*, 2008), *Saccharum officinarum* (Balamurugan *et al.*, 2009), *Piper betle* (Sharma *et al.*, 2007), and *Catharanthus roseus* (Gupta, 2009).

The observation that both fractions had LD₅₀ greater than 5g/kg suggests the safety nature of the fractions (OECD, 2008).

Chemical, biological, or physical assays are necessary to identify the responsible compound(s) from a complex natural product being used in any study. The target compounds may be of certain chemical classes and have

certain physical properties, or possess certain biological activities. Therefore, appropriate assays should be incorporated in the extraction and isolation protocol (Sarker, 2005). In the present study, the phytochemical screening of the aqueous and butanol fractions indicated the presence of phytosterols, polyphenols and tannins which are higher molecular weight polyphenols (Alugah and Ibraheem, 2014) and this could possibly suggest the antifertility effect of the plant. These constituents were also identified and reported in *Ficus asperifolia* (Watcho *et al.*, 2009) and *Alangium salviifolium* (Linn. f.) Wang (Murugan *et al.*, 2000), which are traditionally employed antifertility plants.

Conclusion and Recommendations

The present study indicated that both aqueous and butanol fractions of the methanolic root extract of *Rumex stuedelii* have dose dependent anti-fertility effect. The antifertility effect of the aqueous fraction may be attributed to its anti-implantation activity resulting from an increase in the progesterone hormone and reduced uterine ratio, but that of the butanol fraction might be due to reduced uterine ratio and repeated administration. The antifertility effect is more with the aqueous fraction than with the butanol fraction. Moreover, both fractions employed in the present study seem to be safe. The aqueous fraction were safer than the butanol based on LD₅₀ finding. The polyphenols, phytosterols, and tannins might also be responsible for the anti-fertility activity in both fractions.

In general, comprehensive investigations should be conducted to establish additional mechanism(s) of the action of the aqueous and the butanol fractions such as effects on serum Gonadotropin Releasing Hormone (GnRH), FSH, LH and uterine contractility. Further determination on the quantity of the screened phytochemicals, efficacy, safety, isolation, characterization, structural elucidation of active principles, and toxicity studies on chronic administration on these fractions should be conducted. Moreover the antifertility effects and phytochemical screening of other fractions need to be investigated.

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Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contributions

TG performed the laboratory work, acquired and analyzed the data and drafted the manuscript. EM conceived the research idea. AD identified and provided the experimental plant and facilitated for laboratory work in Ethiopian Public Health Institute. EM and AD participated in the design of the study, correction and approved the final version of the research manuscript.

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