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RESEARCH ARTICLE

Antitumor and Wound Healing Properties of *Rubus ellipticus* Smith.

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Abstract
The present investigation has been undertaken to study the antioxidant, antitumor, and wound healing properties of *Rubus ellipticus*. The *R. ellipticus* leaves were extracted using organic solvents in Soxhlet and were subjected to *in vitro* antioxidant assays. *R. ellipticus* leaf methanol (RELM) extract, which showed higher *in vitro* antioxidant activity, was taken for the evaluation of *in vivo* antioxidant, antitumor, and wound healing properties. Acute oral and dermal toxicity studies showed the safety of RELM up to a dose of 2 g/kg. A significant wound healing property was observed in incision, excision, and *Staphylococcus aureus*-induced infected wound models in the treatment groups compared to the control group. A complete epithelialization period was noticed during the 13th day and the 19th day. A 250-mg/kg treatment was found to prolong the life span of mice with Ehrlich ascite carcinoma (EAC; 46.76%) and to reduce the volume of Dalton's lymphoma ascite (DLA) solid tumors (2.56 cm^3). The present study suggests that *R. ellipticus* is a valuable natural antioxidant and that it is immensely effective for treating skin diseases, wounds, and tumors.

1. Introduction

Wound healing is the body's natural process of regenerating in a wounded area. When an individual is wounded, a set of biochemical events take place, i.e., inflammatory, proliferative, and remodeling phases [1]. The bioactive compound that promotes these events can be therapeutically used to improve the wound healing process. Reactive oxygen species (ROS) play a vital role in wound healing. Superoxide is rapidly converted to H_2O_2 by superoxide

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dismutase (SOD). Release of H_2O_2 may promote formation of other oxidants that are more stable—this suggests that the wound site is rich in oxidants [2]. Antioxidants therefore enhance wound healing by reducing the damage caused by radicals. Hence, plants showing antioxidant properties could also have wound healing activity. The target of many research studies has been the discovery of natural and synthetic compounds that can be used in the prevention and/or treatment of cancer. Several studies have shown that the extracts from different medicinal plants have great significance in reducing the risk of incidence and progression of tumors.

The genus *Rubus* is very diverse. It includes over 750 species in 12 subgenera and is found in all continents except Antarctica [3]. *R. ellipticus* is a weedy raspberry, well established in disturbed wet forests with an elevation of 548—1700 m, and thrives in sunny open pastures as well as deep rain forests [4]. Previously reported as an *in vitro* antioxidant, its traditional uses and pharmacological properties, such as anti-inflammatory, analgesic, and antipyretic activities [5], encouraged us to carry out this study. The *Rubus* species is well known for its pharmacological properties; a survey of the literature revealed that the antioxidant, antitumor, and wound healing potential of this plant has not been fully evaluated. Keeping this in mind, the present investigation was undertaken to study the antioxidant, antitumor, and wound healing activities of *R. ellipticus* leaf and to put forward a scope to develop an effective drug for the treatment of wounds, bacterial infections, and tumors.

2. Materials and methods

2.1. Plant material and extract preparation

Fresh *R. ellipticus* plant was collected from Marayoor Shola forest, Kerala, India and was identified by the Botanical Survey of India (Tamil Nadu, India; voucher specimen No. BSI/SRC/5/23/2010-11/Tech.1659). The powdered leaf was extracted successively in Soxhlet (Borosil Glass Works Limited 1101, Crescenzo, G-Block, Opp. MCA club, Bandra Kurla Complex, Mumbai 400051, India) using organic solvents. The chemicals such as DPPH, Nitric oxide, Superoxide, Tris HCl, SOD, Catalase, GSH and GPx were purchased from Sigma Aldrich Chemicals (Bommansandar Jigani Link Road Industrial Area, Bangalore 560100, Karnataka, India).

2.2. Determination of *in vitro* antioxidant activity

The antioxidant activities of the leaf extracts were determined in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the method of Blois [6]. Nitric oxide [7] and superoxide [8] radical scavenging activities of the *R. ellipticus* leaf extracts were also assayed to confirm its antioxidant potential.

2.3. Determination of *in vivo* antioxidant activity

All of the animal experimental protocol was subjected to scrutiny by the institutional animal ethics committee for experimental clearance (Kovai Medical Center Research and Educational Trust, KMCRET/Ph.D/03/2011). The acute toxicity studies were carried out by following Organization for Economic Co-operation and Development (OECD) guidelines [9]. Thirty Swiss albino male mice were divided into five groups of six animals and treated orally with different doses of *R. ellipticus* leaf methanol (RELM) extract dissolved in 0.1% carboxy methyl cellulose (CMC) for 30 days: Group I, normal; Group II, control treated with 0.1% CMC; Group III, 50 mg/kg body weight (b. wt); Group IV, 100 mg/kg b. wt; and Group V, 250 mg/kg b. wt.

At the end of the experiment, animals were sacrificed, the liver was excised and washed in ice-cold trisaminomethane hydrochloride (Tris—HCl) buffer (0.1 M, pH 7.4), and cytosolic samples of liver homogenate were prepared by centrifugation at 9500 x *g* for 30 minutes at 4°C.

The estimation of total protein was carried out by Lowry's method [10]. The following parameters were assayed in liver to assess the oxidative stress: SOD activity was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich [11]. Catalase (CAT) activity was estimated by the method of Aebi [12] by measuring the rate of decomposition of hydrogen peroxide at 240 nm. Glutathione (GSH) activity was assayed by the method of Moron [13]; assay of glutathione peroxidase (GPX) was carried out by the method of Hafeman [14] based on the degradation of H_2O_2 in the presence of GSH.

2.4. Wound healing activity

Wistar male rats (150—250 g) were used to assess the wound healing property (Rats, mice: Small animals breeding station, College of Veterinary and Animal Sciences, Mannuthy, Trissur, India; Cell lines: Amala Cancer Research Centre, Trissur, India). The experimental protocol was subjected to the scrutiny of the institutional animal ethical committee for experimental clearance. Acute dermal toxicity was checked as per OECD guidelines 404 [15]. The most commonly used trituration method as mentioned in British Pharmacopeia [12] was followed for extract ointment preparation (1% and 2%). The animals were divided into groups (8 animals per group) as follows:

Excision model: Group I, control; Group II, control treated with simple ointment base; Group III, Betadine 5% (w/w); Group IV, RELM 1% (w/w); and Group V, RELM 2% (w/w).

Infected model: Group I, control; Group II, control treated with simple ointment base; Group III, Betadine 5% (w/w); Group IV, RELM 1% (w/w); Group V, RELM 2% (w/w); and Group VI, Neomycin 5% (w/w).

Incision model: Group I, control; Group III, Betadine 5% (w/w); Group IV, RELM 1% (w/w); and Group V, RELM 2% (w/w).

2.4.1. Excision and infected wound models

An excision wound of 1.5 cm in diameter and 0.2 cm depth was created on the shaved dorsal side. In the infected model, 0.1 mL of saline containing 10^6 colony forming units (CFU)/mL of *Staphylococcus aureus* suspension was swabbed on the wounded area. The healing property was evaluated by wound contraction percentage and closure time.

Table 1 Free radical scavenging activities of RELM.

Sample	DPPH radical scavenging activity (IC_{50} μg/mL)	Nitric oxide radical scavenging (%inhibition) (100 μg/mL)	Superoxide radical scavenging (%inhibition) (100 μg/mL)
Petroleum ether	182.48 ± 3.43^{c}	26.54 ± 2.74^{c}	59.23 ± 0.83^{c}
Chloroform	270.27 ± 2.44^{d}	34.09 ± 3.34^{c}	45.34 ± 1.45^{c}
Acetone	7.05 ± 2.02^{a}	70.37 ± 2.36^{b}	65.71 ± 2.07^{b}
Methanol	6.96 ± 2.32^{a}	71.08 ± 3.34^{b}	66.08 ± 2.11^{b}
Hot water	7.39 ± 1.23^{a}	20.01 ± 3.68^{d}	63.44 ± 0.54^{b}
BHT	13.18 ± 1.43^{b}	91.02 ± 1.77^{a}	94.20 ± 2.12^{a}
BHA	4.88 ± 1.45^{a}	—	94.70 ± 1.78^{a}
Rutin	5.81 ± 2.34^{a}	93.24 ± 1.96^{a}	—
Quercetin	4.12 ± 1.67^{a}	—	—

Values are mean of triplicate determinations ($n = 3$) ± standard deviation (SD).
BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC_{50} = an inhibitory concentration 50; RELM = *Rubus ellipticus* leaf methanol.
Statistically significant at $p < 0.001$ where a > b > c > d in each assay.

The granuloma tissue excised from the wound site was used for the estimation of SOD levels and histopathological analysis [16–19].

2.4.2. Incision model

A long incision of 6 cm was made through the full thickness of the skin. The wounds were closed with sutures 1 cm apart using a surgical thread and a curved needle. The drugs were topically applied once daily for 10 days. All the sutures were removed on the 10th day and tensile strength was measured [20]:

$$\text{Tensile strength} = \frac{\text{Breaking force (N)}}{\text{Area (cm}^2\text{)}}$$

where area = thickness × width.

2.5. Effect of RELM extract on solid and ascites tumor development

Dalton's lymphoma ascite (DLA) cell lines (10^6 cells/animal) were injected subcutaneously into the right hind limb of Swiss albino mice for solid tumor development and divided into five groups containing six mice in each group [21]. Group I served as tumor control and received CMC (0.1%). Group II was treated with cyclophosphamide (10 mg/kg b. wt). Groups III, IV, and V were treated with RELM extract at 50 mg/kg b. wt, 100 mg/kg b. wt, and 250 mg/kg b. wt doses, respectively. The treatments were administered orally after 24 hours of DLA cell inoculation and continued once daily for 10 days. The initial diameter of the right hind limb was noted using vernier calipers. The tumor volume (V) was measured every 3rd day and recorded up to 1 month:

$$V = 4/3 \times \pi \times r_1^2 \times r_2$$

where r_1 and r_2 are the radii of tumors in two different planes.

Male Swiss albino mice were injected with EAC cell lines (10^6) intraperitoneally for the development of ascites tumor [22] After 24 hours of tumor inoculation, the animals were treated with different concentrations of RELM extract (50 mg/kg b. wt, 100 mg/kg b. wt, and 250 mg/kg b. wt) and cyclophosphamide (10 mg /kg b. wt) for 10 days. The development of ascites tumors in all the animals were noted and the increase in life span (ILS) of animals was calculated:

$$\%\,\text{ILS} = (T - C)/C \times 100$$

where T and C are the average number of days the treated and control animals survived.

2.6. Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM); analyzed using one-way analysis of variance (ANOVA) and significance was determined by Duncan's, Tukey Kramer multiple comparisons, or Dunnet multiple range test using SPSS version 17 (SPSS Inc., Chicago, IL, USA) and Graphpad (GraphPad Software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA 92037, USA).

Table 2 Effect of RELM on antioxidant enzymes in liver.

Group	Glutathione (nmol/mg protein)	Glutathione peroxidase (U/mg protein)	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)
Normal	10.76 ± 1.08	9.73 ± 1.50	3.64 ± 1.66	0.87 ± 1.23
Control (0.1% CMC)	9.33 ± 1.22	10.28 ± 1.82	3.55 ± 1.03	0.91 ± 2.62
RELM (100 mg/kg b. wt)	11.89 ± 0.53*	12.89 ± 1.55*	4.01 ± 0.70	1.01 ± 0.42
RELM (250 mg/kg b. wt)	$12.78 \pm 0.98^{\dagger}$	$14.09 \pm 1.09^{\dagger}$	5.23 ± 0.55*	1.29 ± 0.93

Values are expressed as mean ± standard error of the mean (SEM; $n = 6$).
Significantly different at *$p < 0.01$, $^{\dagger}p < 0.001$, when compared to control. b. wt = body weight; CMC = carboxy methyl cellulose; RELM = *Rubus ellipticus* leaf methanol.

Table 3 Effect of RELM on excision and *Staphylococcus aureus*-infected wounds.

Groups	Mean percentage of wound contraction ± SEM						Period of epithelialization (days)		SOD activity of granuloma tissue (U/mg protein)	
	Day 3		Day 12		Day 21					
	Excision	Infected	Excision	Infected	Excision	Infected	Excision	Infected	Excision	Infected
Control	12.61 ± 2.31	15.38 ± 3.7	44.00 ± 2.58	23.62 ± 1.6	73.80 ± 2.5	67.29 ± 1.4	27.00 ± 0.03	35.82 ± 0.95	0.87 ± 0.24	0.85 ± 0.76
Betadine (5% w/w)	23.96 ± 2.3*	19.01 ± 2.38	100.0 ± 1.0*	81.20 ± 1.32*	100.0 ± 0.0*	100.00 ± 0.0*	12.62 ± 0.06*	15.01 ± 0.02*	1.28 ± 0.24	1.29 ± 0.54
Neomycin (5% w/w)	—	20.47 ± 2.77	—	100.00 ± 0.02*	—	100.00 ± 0.0*	—	12.06 ± 0.11*	—	1.31 ± 0.35
Ointment base	14.46 ± 1.94	19.87 ± 2.9	56.32 ± 1.25*	38.46 ± 2.1*	82.5 ± 1.70*	75.45 ± 2.4*	23.39 ± 0.82*	29.14 ± 0.84*	0.85 ± 0.15	0.89 ± 0.54
RELM1	14.30 ± 2.54	22.44 ± 1.8†	69.03 ± 1.52*	60.23 ± 1.9*	100.0 ± 0.0*	100.00 ± 0.0*	18.01 ± 0.02*	19.58 ± 0.21*	1.14 ± 0.61	1.08 ± 0.45
RELM2	8.68 ± 1.8	13.27 ± 2.53	94.23 ± 1.9*	79.25 ± 1.09*	100.0 ± 0.0*	100.00 ± 0.0*	13.84 ± 0.09*	15.0 ± 0.00*	1.26 ± 0.49	1.15 ± 0.62

Values are expressed as mean ± standard error of the mean (SEM), $n = 8$ animals in each group.
The treated groups are compared with the control group. *$p < 0.001$, †$p < 0.05$. RELM1, RELM2 = *Rubus ellipticus* root acetone extract of 1% and 2% w/w ointment; SOD = superoxide dismutase.

3. Results

3.1. *In vitro* antioxidant activities of *R. ellipticus*

The RELM extract was found to scavenge the stable free radical DPPH significantly ($p < 0.001$) with an inhibitory concentration 50 (IC_{50}) value of 6.96 μg/mL, which was compared with the standards butylated hydroxytoluene (BHT; 13.18 μg/mL; $p < 0.01$), butylated hydroxyanisole (BHA; 4.88uμg/mL; $p < 0.001$), rutin (5.80 μg/mL; $p < 0.001$), and quercetin (4.12 μg/mL; $p < 0.001$), respectively (Table 1). One hundred μg/mL of RELM extract was found to inhibit 71.08% nitric oxide free radicals when compared to BHT and rutin ($p < 0.001$). RELM significantly inhibited 66.08% of superoxide radicals, which was comparable to the standards BHA and BHT ($p < 0.001$).

3.2. *In vivo* antioxidant activities of RELM

Because the RELM extract showed a strong *in vitro* antioxidant activity, it was taken for evaluation of *in vivo* antioxidant activity. The activity of GSH, GPx, CAT, and SOD were effectively increased in groups treated with 100 mg/kg b. wt and 250 mg/kg b. wt RELM extract compared to untreated control groups. The GSH level significantly increased in both 100 mg/kg b. wt ($p < 0.05$) and 250 mg/kg b. wt ($p < 0.01$) treated groups. GPx, CAT, and SOD levels were found to be increased significantly ($p < 0.05$) in 250 mg/kg b. wt (Table 2).

3.3. Wound healing properties of RELM

The toxicity study shows safety of the extract up to a maximum dose of 2 g/kg. Hence, two doses were selected for this study, i.e., 100 mg/kg and 200 mg/kg. The mean percentage of contraction of the wound area in the excision and infected models was calculated on postwounding Day 3, Day 6, Day 9, Day 12, Day 15, Day 18, and Day 21. In the excision model, the percentage closure of the wound area had significantly increased (from $p < 0.05$ to $p < 0.001$) by the curative effect of both doses (1% and 2%) by decreasing the epithelialization period (18.01 days and 13.84 days, respectively) as evidenced by the shorter period for fall of escher compared to standard (12.62) and control (27.00). The 2% acetone extract-treated groups demonstrated 94.23% contraction on the 12th day, which was close to the contraction value with Betadine (100% on the 12th day; Table 3).

A very rapid contraction of the infected wound was observed in standard, 1%, and 2% RELM-treated groups from the 12th day to the 19th day postsurgery when compared to other groups. On Day 12, the 2% RELM-treated group showed significant ($p < 0.001$) similarity to the 5% Betadine-treated group. On Day 12, the mean percentage of wound contraction of control, vehicle, Betadine, Neomycin, 1%, and 2% RELM-treated groups were 23.62%, 38.46%, 81.20%, 100%, 60.23%, and 79.25%, respectively. A complete epithelialization was noticed on the 12th day for the 2% extract-treated group. The period of complete epithelialization was delayed by 4 days in the 1% treated groups, and for control and vehicle it was delayed by 20

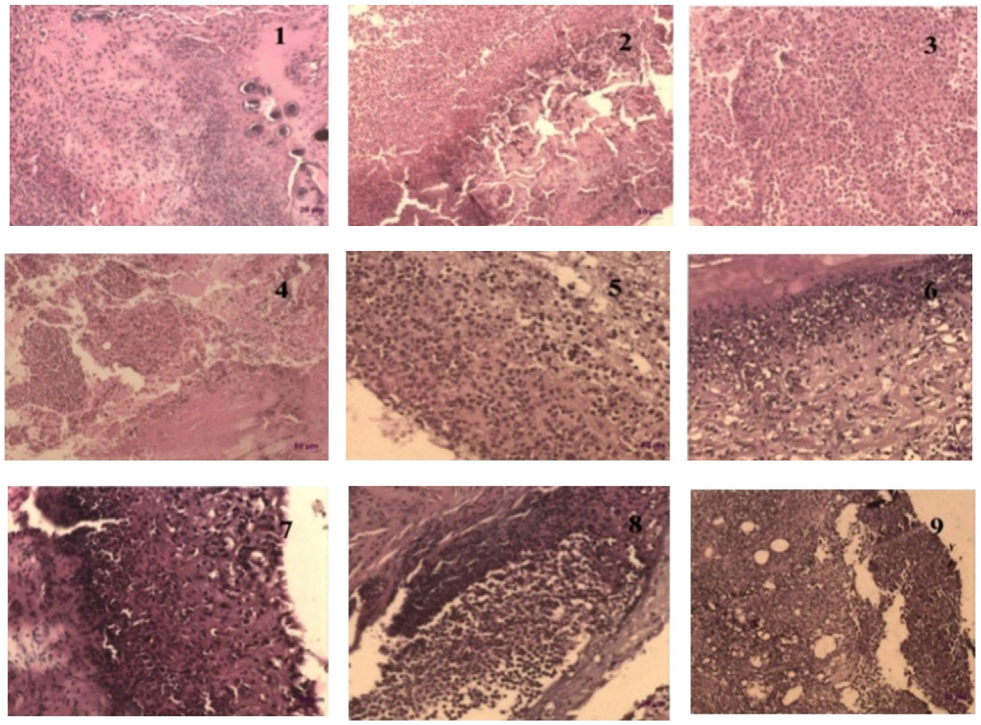


Figure 1 Histopathological view of excision and infected excision wound healing. Epidermal/dermal remodeling in the Day 12 tissue taken from excision model (1, RELM 1%; 2, RELM 2%; 3, control; and 4, Betadine) and infected excision model (5, RELM 1%; 6, RELM 2%, 7, control; 8, Betadine; and 9, Neomycin) animals. Skin sections show the H&E-stained epidermis and dermis. The granuloma tissue section of control rats showing incomplete healing with less epithelialization and lesser collagen formation indicated the incomplete wound healing. Complete healing occurred in the standard and extract-treated rats with elevated epithelialization and increased collagen formation. H&E = hematoxylin and eosin; RELM = *Rubus ellipticus* leaf methanol extract.

days and 14 days, respectively, when compared to the 2% treated group. The SOD activity was found to be slightly increased in all the groups of animals; this indicates a possible mechanism to reduce oxidative stress. Compared to the control groups, SOD levels were significantly increased in 1% and 2% RELM-treated groups.

Hematoxylin and eosin (H&E)-stained slides were evaluated for demonstration of the healing process (Fig. 1). Phases in wound healing processes were observed successfully within the experimental groups. The histopathological evaluation revealed that the original tissue regeneration was much greater in standard and RELM-treated groups.

The tensile strength in the animals of the treatment groups (1% and 2%) were significantly ($p < 0.01$ and $p < 0.001$) greater than that of the control in the incision model. The tensile strength of the animals was 42.45 N/cm^2 (2%), 39.57 N/cm^2 (1%), 67.31 N/cm^2 (Betadine), 70.28 N/cm^2 (normal skin), and 33.50 N/cm^2 (control), respectively (Table 4).

Table 4 Effect of RELM1 and RELM2 on healing of incision wounds.

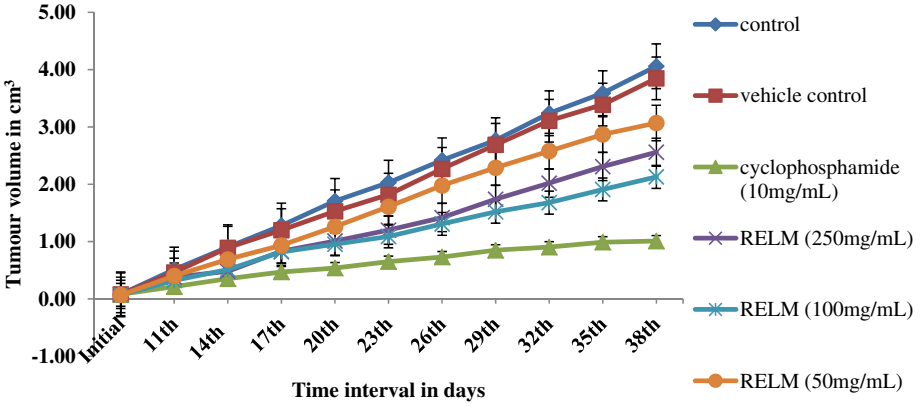
Groups	Tensile strength mean ± SEM (N/cm^2)
Control	33.50 ± 1.2
Standard—Betadine	67.31 ± 1.03*
Normal skin	70.28 ± 0.9*
RELM1	39.57 ± 1.09†
RELM2	42.45 ± 1.34*

Values are expressed as mean ± standard error of the mean (SEM), $n = 6$ animals in each group.
The treated groups are compared with the control group: *$p < 0.001$, †$p < 0.01$. RELM1, RELM2 = *Rubus ellipticus* root acetone extract of 1% and 2% w/w ointment.

3.4. Antitumor activities of RELM

The effect of RELM in the reduction of a solid tumor induced by DLA cells is shown in Fig. 2. The tumor volume of mice treated with RELM was found to be significantly lower than untreated and vehicle (0.1% CMC) controls. The tumor volumes of the untreated and vehicle control animals on the 38th day (10th reading) were 4.06 cm^3 and 3.85 cm^3, respectively, whereas the tumor volumes of the animals treated with 250 mg/kg b. wt, 100 mg/kg b. wt, and 50 mg/kg b. wt RELM extract were 2.56 cm^3, 2.13 cm^3, and 3.07 cm^3, respectively, on the 38th day. RELM also showed a significant reduction of solid tumor volume in Swiss albino mice when injected subcutaneously.

The effect of the RELM extract on ascites tumor development is shown in Table 5. All the control animals died of EAC tumor by 19.16 days. All the treated groups showed an increase in life span of animals bearing EAC tumors. The administration of RELM significantly ($p < 0.001$, $p < 0.01$)



RELM- *Rubus ellipticus* leaf methanol

Figure 2 The effect of *Rubus ellipticus* leaf methanol extract on solid tumor development is shown in the graph. A dose-dependent decrease in the tumor volume was observed; the untreated and vehicle-treated groups showed 4.06 cm^3 and 3.85 cm^3 tumor volume on the 38^{th} day, which was significantly higher than that of the standard and extract-treated groups.

reduced ascites tumor development by EAC cells when injected intraperitoneally. However, cyclophosphamide (10 mg/kg) was found to be more effective and increased the life span of animals by 83.56% ($p < 0.001$) whereas RELM 250 mg/kg, 100 mg/kg, and 50 mg/kg showed 45.76%, 39.93%, and 22.76% increase in life span, respectively.

4. Discussion

Dall' Acqua [23] carried out DPPH method with the leaf methanol extract of *Rubus ulmifolius* to evaluate the antioxidant potential and confirmed that the activity is related to the nature of phenolics; thus contributing to their electron transfer/hydrogen donating ability. A similar effect has been shown by *R. ellipticus* extracts also. The protective effects of *R. ellipticus* fruit on the glucose tolerance test and alloxan-induced diabetes were evaluated [24]. The *R. ellipticus* fruit extracts exhibited a significant antidiabetic effect in experimental models of diabetes mellitus.

Table 5 Effect on *Rubus ellipticus* leaf methanol extract on ascites tumor development.

Groups	Mean survival (days)	Increase in lifespan (%)
Control (tumor alone)	19.16 ± 0.85	—
Vehicle control (0.1% CMC)	20.18 ± 0.92	—
Cyclophosphamide (10 mg/kg)	35.17 ± 1.12†	83.56
R. ellipticus leaf methanol extract (250 mg/kg)	28.12 ± 1.58†	46.76
R. ellipticus leaf methanol extract (100 mg/kg)	26.81 ± 2.01†	39.93
R. ellipticus leaf methanol extract (50 mg/kg)	23.52 ± 1.92*	22.76

Values are expressed as mean ± standard error of the mean (SEM) ($n = 6$).
Significantly different at *$p < 0.01$, †$p < 0.001$ when compared to control. CMC = carboxy methyl cellulose.

The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reaction initiated by the excess generation of NO that is detrimental to human health [25]. Because *R. ellipticus* showed good NO scavenging activity, it is clear that it can be used for scavenging reactive nitrogen species in human body. The human body has several mechanisms to counteract the damage by free radicals; these act on different oxidants as well as in different cellular compartments. One important line of defense is a system of antioxidant enzymes such as SOD, catalase, and GPX. SOD is a metalloprotein, which converts superoxide radicals into H_2O_2. To eliminate H_2O_2, organisms use catalase—a homotetrameric ferri heme containing enzyme and/or GPX, a selenium-dependent enzyme. The K_m value for GPX is lower than that for catalase and hence GPX is considered most important in physiological conditions. GSH is abundant in most cells—it is an important substrate for GPX by quenching free radicals [26]. The results of this study clearly shows that the RELM extract could effectively raise the levels of *in vivo* antioxidants in treated animals, and it may act as a defense system in the human body to counteract oxidative stress.

The aerial parts of *Rubus sanctus* promote wound healing activity in animals as shown in a preclinical study using both incision and excision models [27]. Flavonoids and their derivatives are known to decrease lipid peroxidation by improving vascularity and by preventing or slowing down the progress of cell necrosis. Hence, any drug that inhibits lipid peroxidation is supposed to increase the viability of collagen fibrils by increasing the circulation and strength of collagen fibers, by encouraging DNA synthesis, and preventing cell damage [28]; these factors enhance wound healing. Therefore, the wound healing potential of *R. ellipticus* may be attributed to the phytoconstituents in leaves, which may be either due to their individual or additive effect.

There are reports that extracts from plants such as *Emblica officinalis* [29], *Phyllanthus amarus* [22], *Curcuma longa* [30], *Picrorhiza kurroa* [31] and *Piper longum* [32]

have antitumor, immunomodulatory, and anticarcinogenic activities; all these plants have shown strong antioxidant potential also. These observations suggest that the effect of the RELM extract to inhibit the incidence and progression of tumors is essentially due to its antioxidant activities. The *R. ellipticus* extract was found to have significant activity against DLA- and EAC-induced solid and ascites tumors.

The anti-inflammatory, analgesic, and antipyretic activities of *R. ellipticus* and a closely related species of *Rubus*, *Rubus niveus*, have been reported by George et al [5,33]. Johnston et al [34] studied the acupuncture-enhanced anticancer immune functions of natural killer (NK) cells. The research provides background information on acupuncture, summarizes the current scientific understanding of the mechanisms through which NK cells act to eliminate cancer cells, and reviews evidence that acupuncture is associated with increases in NK cell quantity and function in both animals and humans. Compelling research findings demonstrate that acupuncture reduces the incidence of chemotherapy-induced acute vomiting [35] and potentially manages the cancer-related pain of articular and soft tissue origin [36,37]. Promising evidence suggests that acupuncture relieves fatigue in cancer patients and survivors [38,39]. Therefore, a combinatorial therapy including acupuncture and herbal treatment modalities could impart a synergistic effect on cancer treatment. In this study, the *R. ellipticus* leaf methanol extract processed significant wound healing and appreciable ascites and solid antitumor activities, more than likely due to its strong *in vitro* and *in vivo* antioxidant properties. It may also stimulate the NK cells to elevate the anticancer immune functions of acupuncture-enhanced cancer therapy.

The present study demonstrated that RELM was effective in wound healing and reduction of tumor progression. This may lead to the utilization of *R. ellipticus* as a phytotherapeutic agent for the treatment of free radical-related or -generated disorders.

Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

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