Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(3): 123-128

Received: 15-03-2021
Accepted: 21-04-2021

Samuel Horner
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, MN 55112, USA

Teresa DeGolier
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, MN 55112, USA

Corresponding Author:
Samuel Horner
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, MN 55112, USA

Mitchella repens (Partridge Berry) contracts uterine smooth muscle in isolated mouse tissues

Samuel Horner and Teresa DeGolier

Abstract
Mitchella repens, commonly known as partridge berry, is an herbal that has a long history of effective treatments for a number of female reproductive concerns including the maintenance of a healthy pregnancy, readiness for labor, and successful labor itself. Documentation for it use comes primarily through traditional medicine and midwifery. Very little empirical research has validated the efficiencies of such claims. Thus, the specific objective of this project was to determine whether *Mitchella repens* demonstrated oxytocic activities in uterine tissues. Increasing concentrations of aqueous extracts of *Mitchella repens* were applied directly to isolated mouse uterine horns suspended in an organ bath. Results showed that *Mitchella repens* produced strong contractile responses that were greater than the tissue's own spontaneous motility ($p < 0.0001$), and on average were 80.45% of their own oxytocin positive control. It is likely that the saponin constituent contained within the applied extract is involved with the muscle contractions. The results of this study do provide some empirical support for the claim that *Mitchella repens* can augment labor contractions and/or potentially induce labor. Even though these contractile responses were collected from isolated studies, they may still function to serve as valid indicators of involvement at an organismic level.

Keywords: *Mitchella repens,* labor, smooth muscle contractions, partridge berry, saponins

Introduction

The practice of midwifery blends the wisdom of elders with the science of modern medicine to provide the best holistic care when assisting women in the birthing process [1]. Midwifery care can take place in a variety of locations including a woman's home, a birth center, or a hospital [2]. Midwifery has been observed to provide a viable and safe alternative to physician-delivered maternity care. Births facilitated by midwives are reported to have a lower mortality rate and a greater birthweight when compared with physician attended births [3].

One of the practices of midwives is to include the use of herbals (e.g. blue cohosh *Caulophyllum thalictroides*, black cohosh *Actaea racemose*, red raspberry *Rubus idaeus*, castor bean *Ricinus communis*, evening primrose *Oenothera biennis*) to help induce labor and modulate uterine contractions [4 - 10]. This tradition is attractive as it believed to provide a more natural treatment for delayed labor rather than the usage of pharmaceutically-developed labor inducers that may have harsh side effects [11].

There is a growing list of herbals that have been observed or reported to be extremely effective and beneficial when used properly during pregnancy, labor, and post labor [5, 9, 12, 13]. Frequently, the affirmation or dismissal of such usage comes from a collection of traditions handed down through the ages. Many of these claims are routinely published in the tertiary literature and in online sources without the benefits of demonstrating herbal effectiveness as based on the results of empirically derived data. This lack of research surrounding herbal remedies has resulted in a number of midwife practitioners to call for more systemic investigations *in vivo* or *in vitro* as some contractile herbs may be dangerous to both mother and child [6, 14].

One such herbal that is popularly reported to promote uterine tone and strengthen the uterus for labor, either singly or in conjunction with other herbals, is the leaves and stems of partridge berry *Mitchella repens*. Partridge berry (family Rubiaceae) is an herbaceous woody shrub found in North America [15]. Most of the uses for partridge berry as presented in the public readership are not supported by empirically collected data [12].

The goal of this project was to investigate whether or not an aqueous extract of *M. repens* was able to contract uterine tissues when administered directly to isolated mouse uterine horns.

The specific objectives were to 1) test the null hypothesis that *M. repens* would have no effect on uterine smooth muscle contractility; 2) determine whether the resulting changes in contractile forces were directly proportional to the concentrations of *M. repens* administered, and 3) reflect on which constituents of the aqueous extract may be responsible for the observed biologically responses. This data then, collected *in vitro*, could be considered as empirical support for the claims as put forth by midwifery and oral traditions that *M. repens* can act as an agent to enhance or augment labor.

Materials and Methods

The experimental design employed herein was adapted from earlier published works [16, 17].

Specimens

Eighteen virgin female mice, *Mus musculus* (outbred ICR-CD-1) each weighing 25-30 grams, were obtained from Envigo, Inc. (Indianapolis, Indiana, USA). The mice were kept in Bethel University's animal room and were given sufficient water and food *ad libitum.* All procedures were completed in accordance with the Institutional Animal Care and Use Committee of Bethel University.

Tissue preparation

Twenty-four hours prior to specimen sacrifice, an injection of 0.2 mg diethylstilbestrol (DES) was given to the mice. DES is a synthetic non-steroidal estrogen agonist used to promote mice into the estrus stage of their estrous cycle [18]. It increases the number of gap junctions in the uterine tissue and allows the uterus to contract more effectively as a single unit [19, 20].

The morning of an experiment, a DeJalons Ringer's solution (g/4 L:36 g NaCl, 1.68 g KCl, 2 g $NaHCO_3$, 2 g D-glucose, 0.32 g $CaCl_2$) was made to mimic uterine extracellular fluid. The mice were then euthanized via CO_2 asphyxiation, pinned down to a dissection board, and an incision on the ventral abdomen was made. The uterine horns were then extracted. Each horn received two sutures, each on opposing ends, to stabilize them in an organ bath. One end was tied to a stationary rod, which was anchored into the organ bath, and the other end was tied to a force transducer which was connected to an amplifier and a PowerLab data acquisition system (AD Instruments, Colorado Springs, Colorado, USA). Each uterine horn was left in the organ bath for an hour under 0.8 g of tension [21] and continually aerated (~2 psi) with 95% O_2/5% CO_2. The uterine horn tissues were flushed every fifteen minutes with fresh DeJalons solution. Contractile waveform activities were collected via the PowerLab system.

Testing protocol

After this equilibration period, oxytocin (OXY 10^{-5} M), an endogenous hormone that contracts uterine tissue in the body [22] was applied to the tissues and served as a positive control. The resulting contractions were observed and recorded for 10 min. The tissues were then flushed (i.e. subjected to a washout) and allowed to return to their normal spontaneous rhythm before any *M. repens* treatments were administered. All treatments were pipetted directly onto the tissues, and tissue responses were recorded for 10 min.

Mitchella repens (partridge berry) preparation

Mitchella repens herbals were purchased from Richters Herbals (Goodwood, Ontario, Canada). The plants were finely ground in a coffee grinder and 3.6 grams of ground partridge berry was mixed with 100 mL of boiling deionized water, and allowed to cool. The mixture was then vacuum filtered through Whatman filter papers via a Buchner funnel in an effort to obtain an aqueous extract.

It was found that 1 ml of this filtrate contained 0.3 mg of *M. repens* residue following evaporation of the aqueous component. The concentrations tested herein were 0.045 mg/ml (n=2), 0.09 mg/ml (n=2), 0.19 mg/ml (n=3), 0.375 mg/ml (n=4), 0.72 mg/ml (n=4), and 1.44 mg/ml (n=3). Thus, the varying concentrations of *M. repens* extract given to the muscle tissues were based on the volume of the extract applied to a 20 ml organ bath.

It was beyond the scope of this project to determine which chemical constituents of *M. repens* remained in the aqueous extract, as opposed to those that constrained by the filter paper. It was observed that the applied extract remained water soluble as there was no evidence of a hydrophobic partition settling to the top of the water bath.

Measurements

The resulting collected waveform data was used to measure changes in contractile forces from the baseline tension of the waveform to the maximal force produced within the treatment exposure. To control for the possible force contribution that the tissue's own spontaneous motility might have on the treatments, these forces were also measured in a similar manner before the treatment applications, and were considered as the control, or the "0" treatment.

To normalize for slight variation in the uterine tissue masses, each tissue's maximal contractile response to any given treatment was expressed as a percent of its initial contractile positive control response to 10^{-5} M OXY.

Statistical analyses

The data were summarized as means ± standard error of the mean (SEM) for contractile force for each treatment concentration. Each set of means utilized included tissues 1) with spontaneous motility prior to the application of the treatment; 2) that responded to 10^{-5} M oxytocin; and 3) had a sample size of at least three. One-factor ANOVA tests were used to make comparisons among the means between treatments. Resulting p values ≤ 0.05 were subjected to the Tukey-Kramer post hoc test (JMP 4.0, SAS Institute, Cary, NC) which indicated which treatment (or concentration means) were considered to be statistically different from each other.

Results

Uterine smooth muscle response to oxytocin and *Mitchella repens*

Tissue viability was affirmed by the presence of spontaneous motility at the beginning of an experiment, which produced an average force of 7.79 ± 1.62 mN (n=18). Tissues that did not exhibit spontaneous motility during the pretreatment 60 min equilibration period, did indeed present these endogenous rhythms after the application of oxytocin.

A representative uterine tissue waveform illustrating spontaneous motility as well as responses to both oxytocin and a *M. repens* extract is shown in Figure 1. Oxytocin generated an immediate increase in contractile force (34.0 mN) followed by a sustained plateau response that gradually declined over time. These tissues usually needed to be flushed twice over 20 min to ensure that the contractile tension returned close to baseline prior to adding the experimental treatment, as partially contracted tissue would have been a confounding variable.

The general contractile response from the *M. repens* applications was to produce a rapid increase in contractile force, but in contrast to oxytocin, these responses did not maintain a plateau shape. Instead, they exhibited a slight increase in contractile frequency which eventually returned to pre-application rates. Many of the *M. repens*-induced waveforms eventually resulted in a basal tonus that was maintained slightly higher than the originally baseline before *M. repens* treatment (Fig. 1).

Even though the uterine contractions produced by *M. repens* demonstrated large increases in contractile force as well as modest increases in contractile rates and basal tonus, only the changes in contractile forces are reported herein as they were the most repeatable.

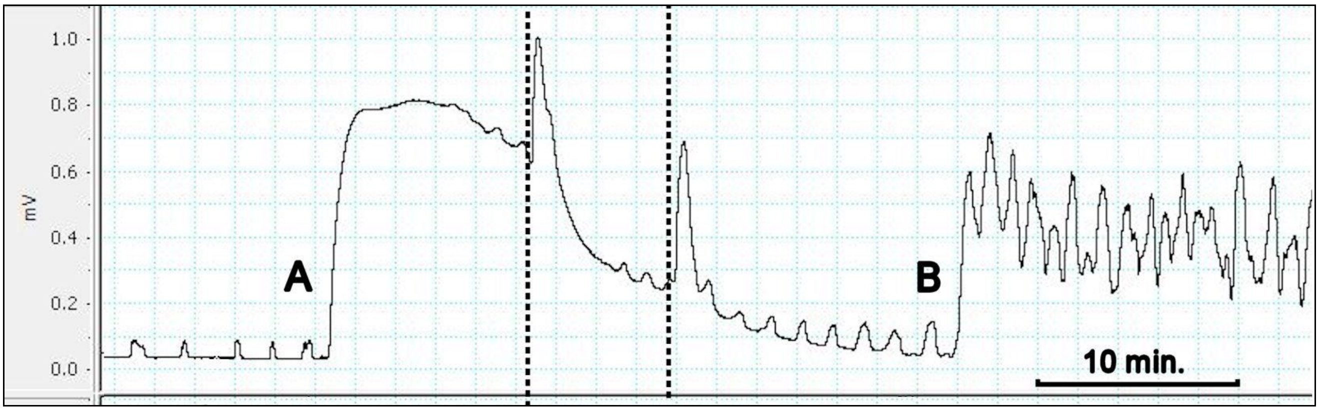

Fig 1: Typical uterine smooth muscle waveform before and after an application of 10^{-5} M oxytocin, followed by a *Mitchella repens* (partridge berry) treatment. In this sample, the observed oxytocin (**A**) generated an increase in contractile force (34.0 mN). Two tissue bath washouts (vertical dotted lines) occurred before a 0.19 mg/ml *M. repens* application was given at letter (**B**). This resulted in an immediate increase in contractile force (29.5 mN), though it was not sustained as long as the contraction produced by oxytocin. The average spontaneous motility before treatment was 3.5 mN. The vertical axis represents the contractile force in mV, later converted to mN.

Uterine smooth muscle response to increasing concentrations of *M. repens*

The average contractile forces (mN) produced from the *M. repens* treatments (mg/ml) were as follows: 0.045 mg/ml: 8.14 mN, 0.09 mg/ml: 11.91.55 mN, 0.19 mg/ml: 25.45 ± 6.76 6mN, 0.375 mg/ml: 35.36 ± 7.51 mN, 0.72 mg/ml: 23.98 ± 7.13 mN, and 1.44 mg/ml: 25.34 ± 1.70.

Increases in contractile forces were observed from concentrations of *M. repens* of 0.19 mg/ml and greater (Fig. 2). These same increases in uterine contractile force production were statistically greater than the “0” treatments, the tissue’s own spontaneous motility ($p < 0.0001$) and on average were 80.45 ± 6.12 % of their oxytocin responses (n=14). The minimal concentration used to achieve the maximal response was 0.375 mg/ml.

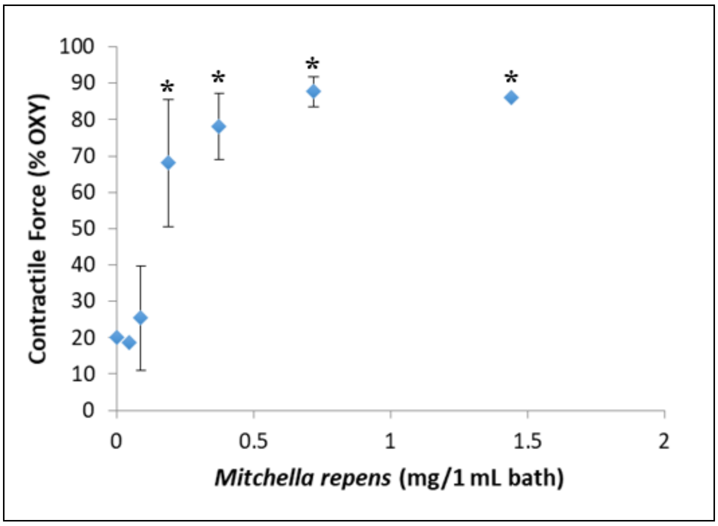

Fig 2: Means ± SEM of uterine contractile force (% OXY) in response to increasing extract concentrations of *Mitchella repens*. The uterine contractile responses produced by the 0.19 mg/ml, 0.375 mg/ml, 0.75 mg/ml, and 1.44 mg/ml treatments (*) were statistically greater than the “0” treatment, the tissue’s own spontaneous motility ($p < 0.0001$)

Discussion

Mitchella repens produced large contractile forces in the isolated uterine tissues from concentrations of 0.19 mg/ml and greater. These results support the hypothesis that the herbal *M. repens*, applied as an aqueous extract, does produce an oxytocic response when applied directly onto uterine tissues. These contractions were also statistically more forceful than the tissue’s own spontaneous motility ($p < 0.0001$). This data then, collected *in vitro*, does serve to provide some empirical support for the claims as put forth by midwifery and oral traditions that *M. repens* may be used as an agent to enhance or augment labor.

A knowledge of the biologically active constituents found in a *M. repens* extract can be helpful in elucidating the mechanism by which *M. repens* induces contraction in uterine smooth muscle. Saponins are secondary metabolites that have been reported in several different plant species [23]. They are often found to be plant specific with variable distributions within a plant [24]. Several publications agree that *M. repens* does contain triterpenoid saponins [9, 13, 25-29]. These studies however, do not report on the distinctive characterizations of the saponins as based on their unique triterpenoid 30 carbon pre-curser skeleton molecules [30]. Other species examined from same family as *M. repens* (Rubiaceae), are also reported to also contain terpenoids [31, 32].

Saponins are one probable constituent which contributes to the process by which contractions are induced. Previous research has demonstrated that saponins isolated from the soap tree *Quillaja saponin* and applied to isolated uterine smooth muscle, does produce powerful contractions [16]. Saponins are considered to be cell permeating agents [33-35] and are thought to disrupt the lipid bilayer by forming pores in the membrane [36, 37]. This pore formation is then thought to be responsible for allowing an influx of extracellular Ca^{2+} ions to initiate the calcium-calmodulin complex and begin the contractile cascade that ensues.

The prolific presence of saponins in plants has also received considerable utilization in traditional medicine. This constituent has been reviewed and is reported to display antimicrobial, antifungal, antiviral, and antioxidant as well as insecticidal and molluscicidal actions [31, 38]. It would seem logical then that the saponin constituents found in *M. repens* would also contribute to its reported therapeutic effects for several physiological concerns unrelated to reproductive function. Traditional medicine, folklore, and several collections of web pages and related content recommend *M. repens* as treatment for urinary tract infections, interstitial cystitis, diarrhea, irritable bowel syndrome, inflammatory bowel syndrome colitis, benign prostate hypertrophy, and rheumatism. Many of the reported benefits summarize subjective observations or experiences that can be influenced by personal feelings. A consistency of positive outcomes without any negative consequences frequently leads to the opinion that *M. repens* is an effective and safe therapeutic. This philosophy has often hindered the motivation and financial support needed to investigate such claims by means of testable hypotheses.

Much less research has been reported on the presence and activities of other *M. repens* phytochemical constituents. The presence of alkaloids has been reported [26] as well as the presence of tannins [28]. Even without a more thorough knowledge of constituent biological activity, *M. repens* has been demonstrated to have some cytotoxic and anti-tumor activity against malignant neuroblastoma *in vitro*. With the goal of screening a number of international medical herbals for their tumoricidal potency, *M. repens* has been rated as a category 2 (1 strongest – 5 weakest) for a possible chemotherapy agent [39]. Other studies testing saponins from other herbals have indeed shown that they may are effective in suppressing cancer-like behaviors in a number of cell lines [40-43].

Figure 2 shows how the concentration of *M. repens* influenced the magnitude of the uterine smooth muscle contractile responses. Additional research with the goal of collecting contractile responses evoked from concentrations between 0.09 mg/ml and 0.19 mg/ml would provide a more informative concentration-response curve. Even so, using the data collected herein, the *M. repens* concentration that gave half the maximal response was estimated to be 0.21 mg/ml. As can be seen from Fig. 2, administering greater than 0.72 mg/ml of the *M. repens* extract is not likely to produce a more forceful contraction. One reason for this may be that the contractile mechanism is already running in a saturated manner. But it can also be speculated that supramaximal concentrations might yield a hysteresis curve, in which the previously observed maximal contractions are suppressed due to potential attenuating interactions among the herbal constituents or their metabolites.

Concentration-response correlations are also important when considering effective dosing with combined herbals. A number of uterine toning, miscarriage preventative, and pregnancy formulas [5, 13, 44, 45, 46] include *M. repens* as an adjunct with other herbals that have also been shown to demonstrate oxytocic behaviors. From the published literature, such herbals include blue cohosh *Caulophyllum thalictroides* [47], red raspberry *Rubus idaeus* [48], cramp bark *Viburnum opulus* and black cohosh *Actaea racemose* [49]. Knowing the summative responses of effective treatment constituent concentrations can favor a healthy timely labor or an unfortunate miscarriage or abortive response.

While the results presented herein convincingly show that *M. repens* induces contractions in uterine smooth muscle tissue *in vitro*, further information is warranted in determining whether or not the same results can be seen in *vivo* [50] when the herbals are ingested as water, spirit, or oil-based medicines [4]. While the consumption of *M. repens* has been considered safe at recommended practitioner schedules [5, 12], little is actually known about its absorption, bioavailability, distribution to the uterus, metabolism, and excretion. While the application at an organismal level may be limited, responses collected from isolated studies may still function to serve as valid indicators [50].

Conclusion

The specific outcome of this project demonstrated that aqueous extracts of *Mitchella repens* contracted smooth muscle from isolated uterine tissues. These results support the hypothesis that the herbal does result in an oxytocic effect when applied directly onto uterine tissues. It is proposed that the saponin constituents are involved with the smooth muscle contractions. This study presents some empirical support that *M. repens* can augment or potentially induce labor. Even though these contractile responses were collected from isolated studies, they may still function to serve as valid indicators of involvement at an organismic level.

References

1. Richards LB. Midwifery - the second oldest profession. International Journal of Childbirth Education 1995;10(2):11-13.
2. Stark M, Remynse M, Zwelling E. Importance of the birth environment to support physiologic birth. Journal of Obstetric, Gynecologic and Neonatal Nursing 2016;45(2):285-294.
3. MacDorman M, Singh G. Midwifery care, social and medical risk factors, and birth outcomes in the USA. Journal of Epidemiology and Community Health 1998;52:310-317.
4. Weed S. Herbal for the Childbearing Year. Wise Women. Ash Tree Publishing, Woodstock, New York 1986, 171s.
5. Belew C. Herbs and the childbearing years. Journal of Nurse-Midwifery 1999;44(3):231-252.
6. McFarlin B, Gibson M, O'Rear J, Harman P. A national survey of herbal preparation use by nurse-midwives for

labor stimulation. Journal of Nurse-Midwifery 1999;44(3):205-216.
7. Hardy M. Women's health series: Herbs of special interest to women. Journal of the American Pharmaceutical Association 2000;40(2):234-242.
8. Dennehy C, Tsourounis C, Bui L, King T. The use of herbs by California midwives. Journal of Obstetric, Gynecologic, and Neonatal Nursing 2010;39:684-693.
9. Shinde P, Patil P, Bairagi. Herbs in pregnancy and lactation: A review appraisal. International Journal of Pharmaceutical Sciences and Research 2012;3(9):3001-3006.
10. Zhang R, Liu Z, Yang D, Zhang X, Sun H, Xiao W. Phytochemistry and pharmacology of the genus *Leonurus*: The herb to benefit the mothers and more. Phytochemistry 2018;147:167-183.
11. Steinberg D, Beal M. Homeopathy and women's healthcare. Journal of Obstetric, Gynecologic, and Neonatal Nursing 2003;32:207-214.
12. Westfall R. Herbal medicine in pregnancy and childbirth. Advances in Natural Therapy 2001;18(1):47-55.
13. Noe J, Bove N, Janel K. Herbal tonic formulas for naturopathic obstetrics. Alternative and Complementary Therapies 2002;8(6):327-335.
14. Panganai T, Shumba P. The African Pitocin - a midwife's dilemma: the perception of women on the use of herbs in pregnancy and labour in Zimbabwe, Gweru. The Pan African Medical Journal 2016;25:9. Published 2016 Sep 19. doi:10.11604/pamj.2016.25.9.7876.
15. Niering W, Olmstead N. National Audubon Society Field Guide to North American Wildflowers, Eastern Region. Chanticlear Press Edition. Alfred A. Knopf, New York. 1998.
16. Bristol B, DeGolier T. *Quillaja* saponins are a potent contractor of uterine smooth muscle tissue *in vitro*. Journal of Pharmacognosy and Phytochemistry 2018;7(5):1252-1258.
17. DeGolier T, Sam Adamson. Aqueous extracts of clary sage (*Salvia sclarea)* contract isolated strips of mouse uterine tissue. Journal of Pharmacognosy and Phytochemistry 2021;10(2):59-64.
18. Allen E. The oestrus cycle in the mouse. American Journal of Anatomy 1992;30:297-371.
19. Doherty L, Bromer J, Yuping Z, Tamir A, Hugh T. *In utero* exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: An epigenetic mechanism linking endocrine disruptors to breast cancer. Hormones and Cancer 2010; 1(3):146-155.
20. Burger H, Healy D, Vollenhover B. The ovary: basic principles and concepts. *In*: Felig P, Rohman L, editors. Endocrinology and Metabolism. 4th ed. USA: McGraw-Hill, Inc 2011, 769.
21. Kitchen I. Textbook of *in vitro* practical pharmacology. Blackwell Scientific Publication. London 1984, 33.
22. Husslein P, Fuchs AR, Fuchs F. Oxytocin and the initiation of human parturition: I. Prostaglandin release during induction of labor by oxytocin. American Journal of Obstetrics and Gynecology 1981;141(5):688-693.
23. Hostettmann K, Marston A. Saponins. Chemistry and pharmacology of natural products. Cambridge University Press, Cambridge 2005.
24. Biswas T, Dwivedi U. Plant triterpenoid saponins: Biosynthesis, *in vitro* production, and pharmacological relevance. Protoplasma 2019;256:1463-1486.
25. Breneiser E. Constituents of some American plants. Abstracts from Ph D Theses. American Journal of Pharmacy (1835-1907); Philadelphia 1887, 228.
26. Chandler F, Hooper S. Herbal remedies of the maritime Indians: A preliminary screening: Part III, Journal of Ethnopharmacology 1982;6(3):275-285.
27. Hooper S, Chandler R. Herbal remedies of the maritime Indians: Phytoesterols and triterpenes of 67 plants. Journal of Ethnopharmacology 1984;10:181-194.
28. Beck B. Ethnobotanical studies of *Mitchella repens* L. (Rubiaceae). Ph D Dissertation Miami University, Oxford, Ohio 1999.
29. Weed S. Planting the Future: Saving Our Medicinal Herbs, 2000 - books.google.com
30. Vincken J, Heng L, de Groot A, Gruppen H. Saponins, classification and occurrence in the plant kingdom. Phytochemistry 2007;68(3):275-297.
31. Sparg SG, Light ME, Van Staden J. Biological activities distribution of plant saponins. Journal of Ethnopharmacology 2004;94:219-243.
32. Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawats S *et al.* Antimicrobial and antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. Molecules 2008;13:904-921.
33. Hirata M, Hirata M, Kukita M, Kukita M, Sasaguri T, Sasaguri T *et al*. Increase in Ca^{2+} permeability of intracellular Ca^{2+} store membrane of saponin-treated guinea pig peritoneal macrophages by inositol 1,4,5-trisphosphate. Journal of Biochemistry 1985;97(6):1575-1582.
34. Kargacin M, Kargacin G. Direct measurement of Ca^{2+} uptake and release by the sarcoplasmic reticulum of saponin permeabilized isolated smooth muscle cells. Journal of General Physiology 1995;106(3):467-484.
35. Berlowska J, Dudkiewicz M, Kregiel D, Czyzowska A, Witonska I. Cell lysis induced by membrane-damaging detergent saponins from *Quillaja saponaria*. Enzyme Microbial Technology 2015;75-76:44-48.
36. Bangham A, Horne R. Action of saponins on biological cells membranes. Nature 1962;196:952-953.
37. Das T, Banerjee D, Chakraborty D, Pakhira M, Shrivastava B, Kuhad R. Saponin: Role in animal system. Veterinary World 2012;5(4):248-254.
38. Bergeron C, Marston A, Gauthier R, Hostettmann K. Screening of plants used by North American Indians for antifungal, bactericidal, larvicidal and molluscicidal activities. International Journal of Pharmacognosy 1996;34(4):233-242.
39. Mazzio E, Soliman K. *In vitro* screening of tumoricidal properties of international medicinal herbs: Part II. Phytotherapy Research 2010;24(12):1813-1824.
40. Mbaveng A, Ndontsa B, Kuete V, Nguekeu Y, Çelik I, Mbouangouere R *et al*. A naturally occurring triterpene saponin ardisiacrispin B displayed cytotoxic effects in multi-factorial drug resistant cancer cells via ferroptotic and apoptotic cell death. Phytomedicine 2018;43:78-85.
41. Wang X, Su G, Zhao C, Qu F, Wang P, Zhao Y. Anticancer activity and potential mechanisms of 1C, a ginseng saponin derivative, on prostate cancer cells. Journal of Ginseng Research. 2018;42(2):133-143.
42. Teng J, Qin D, Mei Q, Qiu W, Pan R, Xiong R, *et al.* Polyphyllin VI, a saponin from *Trillium tschonoskii* Maxim. induces apoptotic and autophagic cell death via the ROS triggered mTOR signaling pathway in non-small

cell lung cancer. Pharmacological Research 2019;147:104396.

43. Cao J, Zhao X, Ma Y, Yang J, Li F. Total saponins from *Rubus parvifolius* L. inhibits cell proliferation, migration and invasion of malignant melanoma *in vitro* and *in vivo*. Bioscience Reports 29. 2021;41(1): BSR20201178. doi: https://doi.org/10.1042/BSR20201178
44. Goguen R. c2004. [accessed 2021 March 31] Rebecca's Herbal Apothecary and Supply: Herb article Partridge Berry https://www.rebeccasherbs.com/pages/herb-article-br-partridge-berry.html
45. Barton D. c2018. Natural Fertility Info: Fertility Herb Partridgeberry for Miscarriage Prevention and More [accessed 2021 March 31]. https://natural-fertility-info.com/fertility-herb-partridge-berry-for-miscarriage-prevention-more.html
46. Blankespoor J. c2021 Chestnut School of Herbal Medicine: Partridgeberry Materia Medica [accessed 2021 March 31] https://chestnutherbs.com/partridgeberry-materia-medica/
47. Berger J, DeGolier T. Pharmacological effects of the aqueous extract of *Caulophyllum thalictroides* (blue cohosh) on isolated *Mus musculus* uteri. BIOS 2008;79(3):103-114.
48. Olson A, DeGolier T. Contractile activity of *Rubus idaeus* extract on isolated mouse uterine strips. BIOS 2016;87(2):39-47.
49. Neuenschwander C. Investigation into the use of a CNM Herbal Inducer containing *Actaea racemosa*, *Leonurus cardiaca*, *Mitchella repens*, and *Viburnum opulus* as applied to isolated *Mus musculus* uterine strips. BS Thesis Bethel University, St. Paul, Minnesota 2018.
50. Lin J. Applications and limitations of interspecies scaling and *in vitro* extrapolation in pharmacokinetics. Drug Metabolism and Disposition 1998;26:1202-1212.