

# Insights Into the Ethnopharmacological Features of Purple Pitcher Plant

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## ABSTRACT

The plant *Sarracenia purpurea* belong to the Sarraceniaceae family, it is popularly known as “purple pitcher plant”. *Sarracenia purpurea* is a carnivorous plant which is dependent on insects or protozoan for nutrients. A mature pitcher leaf of *Sarracenia purpurea* is usually green to red in colour with dark-colored veins. Species richness increased with pitcher density, showing that there is a wider variety of species attracted to pitchers in higher density groups than in low density groups. More pitchers in a group may increase the attractiveness of the site, potentially drawing in more insects and leading to natural variation in species. Pitcher leaves of *Sarracenia purpurea* develop through cell division patterns of adaxial tissues that are different from those in bifacial and peltate leaves. The phytochemistry of plant reveals presence of (+)-catechin, morroniside, goodyeroside, Quercetin, quercetin-3-O-galactoside, quercetin-3-O-arabinoside, taxifolin-7-O-galactoside, gossypetin-3-O-galactoside, taxifolin, tamarixetin-3-O-galactoside, betulinic acid, and Ursolic acid of *Sarracenia purpurea*. The *Sarracenia purpurea* is known to show Anti-diabetic activity, Anti-herpes virus activity, Anti-mycobacterial activity, Cytoprotective activity, Local analgesic effects and Anti-viral activity. The present review focuses on pharmacological activities of *Sarracenia purpurea*.

**Keywords:** *Sarracenia purpurea*, Sarraceniaceae, Pitcher plant, Pharmacological activity, Prey capture.

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## INTRODUCTION

Carnivorous plants grow principally in tropical areas of the world. These are the type of plants which derive most of their nutrients from animals primarily insects or protozoan. They are said to be one of those group of plants that do not seem to be self-dependent.<sup>1</sup> There are different types of trapping mechanisms such as pitfall traps, flypaper traps, snap traps *etc.*, that decide the kind of secretion of these plants used for trapping insects or any other animal for consumption.<sup>1</sup> The purple pitcher plant, *Sarracenia purpurea* L. (Sarraceniaceae), is a perennial carnivorous herb that is distributed across Eastern seaboard and Gulf Coast of the United States and Canada. They grow mainly in the areas where soil is skinny layered or poor in nutrients. A mature pitcher leaf of *Sarracenia purpurea* is usually green to red in colour with dark-colored veins. When the pitcher leaves are mature, the reddish colors and markings are visible on the leaves of plant and attractive to some insects and contrast with the predominantly green colors of the vegetation where pitcher plants grow. (Figure 1) Consequently, insects can see pitcher plants at a distance and are attracted to them. By consuming N<sub>2</sub>

from insects trapped within their pitchers (fused leaves), they adapt to nitrogen-poor environments like bogs and peatlands. Thanks to this uncommon natural history, *S. purpurea* has received considerable attention from an ecological perspective however, despite a protracted history of use as an ancient herbal medicine across the continent, the therapeutic potential of the species remains mostly uninvestigated. Throughout the nineteenth century, *S. purpurea* served as a treatment for small pox and more recently, as an injected pain reliever marketed as Sarapin, an alkaline extract of the root that specifically blocks C-fibre excitation.<sup>2</sup> There are completely different secretory glands like alluring glands, mucilage glands and digestive glands that are helpful in trapping insects additionally different microbes that are essential for their survival. In figure 2, it is seen that a pitcher of the leaf is filled with digestive liquid. This plant has been used in traditional medicine for a good sort of medical diseases, like pox infection, gynaecological issues, diabetic issues, mycobacterial infection, and liver/kidney complaints.<sup>3</sup> The taxonomical classification of purple pitcher plant is given in table 1.

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Figure 1: Leaves of *S. purpurea*



Figure 2: A pitcher filled with digestive liquid

Table 1: Taxonomical classification of *S. purpurea*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Ericales
Family	Sarraceniaceae
Genus	Sarracenia
Species	purpurea

**Chemical composition of *S. purpurea*:** The ethanol extract of *S. purpurea* was subjected to chromatographic purification to afford some new compounds. In addition, some other known compounds were obtained and identified as (+)-catechin, morroniside, Quercetin, Chlorogenic acid, Vanillin Caffeic acid, Ellagic acid, goodyeroside, quercetin-3-O-galactoside, quercetin-3-O-arabinoside, taxifolin-7-O-galactoside, gossypetin-3-O-galactoside, taxifolin, tamarixetin-3-O-galactoside, betulinic acid, and Ursolic acid, betulinaldehyde, betulinic acid,  $\beta$ -Sitosterol.<sup>4</sup>

**Effect of color, size, and density of *Sarracenia purpurea* on prey capture**

Two most rich insects, Hymenoptera and Diptera are found in *S. purpurea* pitcher leaves. Pitcher plant attractiveness is analysed by studying following characteristics of *S. purpurea*: Pitcher density, amount of red venation and size of pitcher, including keel width, mouth diameter and pitcher length. After study all these parameters, it is concluded that species richness increased with pitcher density, showing that there is a wider variety of species attracted to pitchers in higher density groups than in low density groups. More pitchers in a group may increase the attractiveness of the site, potentially drawing in more insects and leading to natural variation (and most likely an increase) in species. It is interesting that the same correlation was not found between biomass and pitcher density. On the other hand, it is inaccurate to assume that an increase in species richness also corresponds to an increase in biomass. This model also does not include the species richness of partially digested insects found in the pitcher. Future studies that quantify insect parts as well as whole insects will show a more complete picture of how species richness varies among different densities of pitcher groups. Another limitation to account for in the biomass findings is that this study did not have a regulation for time. Variation in digestion times among pitchers, including length of time it takes to digest.<sup>5</sup> Researches stressed the importance of pitcher plant inhabitants, sunlight, temperature and their impact on plant metabolism, respirations and digestion rates. Future studies that include these factors might be helpful in creating an overall scene of the mechanisms influencing biomass intake. Species richness and biomass showed a significant, positive correlation with mouth diameter. It is also found that with *S. alata* Wood, concluding that larger capture areas (i.e., mouth diameter) should result in more prey captured. But this contrasts the results found by Newell and Nastase

(1998) that showed no correlation between prey capture and pitcher size, namely mouth diameter. In addition, there is no correlation between species richness with biomass and pitcher color.<sup>6</sup> This could be due to the minimal color variation found at study. Green and Horner (2007) found no effect of color variation on prey capture while Newell and Nastase (1998) found that prey was more likely to visit pitchers with more red venation. Here, it is also did not find correlations between species richness or biomass and pitcher length or keel width, which supports other studies with similar conclusions. Keel width is a function of the amount of N<sub>2</sub> intake from prey already inside the pitcher, and not necessarily a determinant in the amount of prey captured. Contradicting results such as these shows how little is known about the exact mechanisms that attract potential prey to pitcher plants, and it may mean that these mechanisms are environment specific.<sup>7</sup>

**Cell division shapes carnivorous pitcher leaves of common pitcher plants**

Mature carnivorous plant leaves of *S. purpurea* is consists of a tube, a keel and a sheath (Fig. 3a, b). Within the tube, vascular tissue bundles purpose towards the outer surface and xylem points towards the inner surface (Fig. 3c, d), indicating that this structure is biface, just like to the blades of typical, placoid leaves. Within the keel, vascular tissue bundles purpose towards the outer surface however, xylem vessels face one another (Fig. 3d), indicating that the keel forms a definite structure from the biface tube. It is investigated the first development of *S. purpurea* pitcher leaves, exploitation scanning microscopy. The adaxial surface of the early leaf anlage is flat (Fig. 3e, f), just like that in typical biface leaves. Once an anlage becomes B100 millimeters long, associate degree an adaxial ridge connecting each side of a leaf margin seems within the middle of the anlage (Fig. 3g), that is analogous to the 'cross zone' protrusions in shield-shaped leaves of *T. majus*11 and pitcher leaves of *D. californica*15. In *S. purpurea*, the ventral ridge develops into a keel (Fig. 3a, b). Once the anlage reaches B200 millimeters long, it becomes obvious that the proximal and distal components of the ventral ridge can type a keel and also the ventral side of the tube, severally (Fig. 3h). As a results of growth within the leaf margin and also the ventral ridge, a hollow structure develops within the distal a part of the anlage (Fig. 3i) and also the continuing growth of those regions deepens the hollow to make a pitcher form (Fig. 3j).<sup>8</sup>



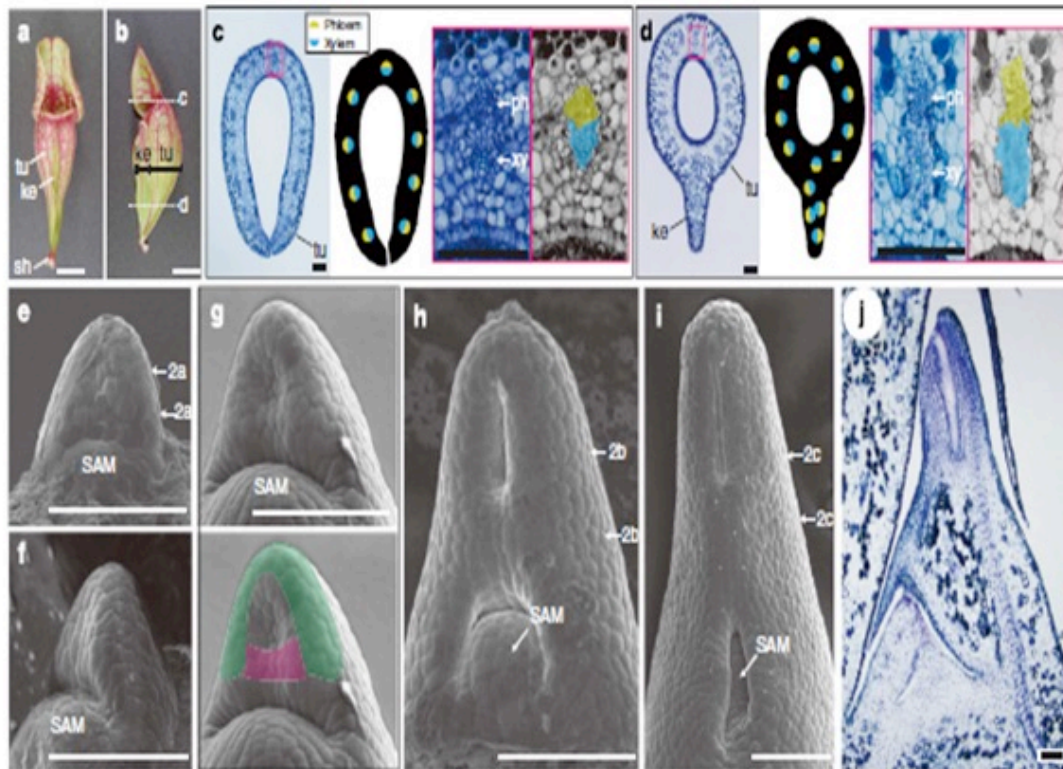


Figure 3: Morphology of *S. purpurea* pitcher leaves.<sup>8</sup>

In this study pitcher leaf development in *S. purpurea* and located that the pitcher shape is established through differential cell division patterns between the hollow and ridge regions of a leaf anlage. The morphology of the pitcher anlage and also the expression patterns of PHB and FIL orthologues before formation of the hollow are just like to those of typical bifacial leaves and shield-shaped leaves during the early developmental stages. Subsequently, pitcher morphology is established through differential cell division patterns in the leaf primordium. In the hollow part of the leaf primordium, longitudinal cell divisions predominated in L1, L2 and L3 cells of both the adaxial and abaxial surfaces, as in conventional bifacial leaves. By contrast, in the ridge region, periclinal cell divisions predominated in the L2 and L3 cells of the adaxial surface and resulted in a protruding ridge that formed a keel. The different mechanisms of growth between the hollow regions and ridge regions form a tube structure.<sup>9</sup> Therefore, the spatial regulation of oriented cell divisions in the leaf primordium is important for pitcher formation. Here used computer simulation to examine the effect of growth parameters because of difficulty in experimental manipulation of multicellular dynamics in planta. In addition to cell division orientation resulting in the specific morphology of hollow and ridge regions, initial morphology and spatial distribution of cell division frequency appear to contribute to leaf development.<sup>10</sup>

#### Metabolic Profiling of *Sarracenia* Carnivorous Plants:

Sarraceniaceae, a plant family mainly comprising of three genera namely, *Darlingtonia* Torr, *Heliamphora*

*Benth*, *Sarracenia* L. The Sarraceniaceae members are characterized by the presence of large number of diverse metabolites [approximately 600 metabolites] in lids as well as pitchers. Coniine is a metabolite that was recently found in seven sarracenia species. Integrating the polygenetic information of Sarraceniaceae, the study done by Hannu Hotti 1 *et al.*, concluded that the metabolic composition of the plant can be demonstrated by the phylogeny which explains the absence and presence of the compounds.<sup>1</sup> The metabolic profiles lids and pitcher are analysed separately, concluding that each plant lid and the pitcher contains about 48 compounds each in total. The biosynthesis of coniine enhances the insect attraction and retention.<sup>11</sup>

#### Pharmacological studies

A number of studies have been carried out on *Sarracenia purpurea* in recent years showing that it possesses diverse pharmacological actions. Some of the important pharmacological actions are as follows:

##### 1) Anti- cancer activity

Cancer is caused by abnormal growth of cells which spreads to all parts of the body in very less time. There are around 100 types of cancer diseases out of which one fourth of cancer deaths are due consumption of tobacco by humans. Infection caused in the body may also become one of the symptoms of cancer. Very high amount of consumption of alcohol may also cause cancer. Lung cancer, cervical cancer and colorectal cancer are one of the most common types of cancers which are highly affecting humans.<sup>12</sup> Almost 15% of deaths out of total cancer patients are occurring every year. Radiation therapy, chemotherapy is helping only

one part of people who can afford financially and others who cannot have no other option but death. This is the present scenario of cancer patients which we are encountering in the World. Immunotherapy has been developing to a larger extent from past decade. New study identifies that cell are being detected with weak immune response before the initiation of treatment for cancer cells with the aid of biomarkers. This research helped inhibitors to activate weak immune cells which would detect cancer cells as foreign bodies and would try to eliminate them. Cytometry by time of flight (CyTOF) cell analysis method helps in analysing 50 different proteins in each cell at a time and thus this helps in identifying activation status of every cell of our body. Immunotherapy analysis helps in early treatment for patients suffering with melanoma and lung cancer.<sup>1</sup> Pan beta blockers which are less prescribed and helps in treatment of heart attacks and also in prevention of lowering blood pressure in patients when given during immunotherapy helps in effectively eradicating melanoma cancer cells. This study states that patients who were treated with immunotherapy along with beta blockers lived longer than those patients who were treated only with immunotherapy. Radiation therapy when given to the patients in different doses help in reducing 20% of side effects caused due to this therapy which could not be reduced by conventional radiation therapy. Fractionation is the principles which are involved in reducing side effects of radiation therapy which are helpful in preventing cancer cells. Insectivorous plants have been found to be rich in secondary metabolites that are more useful in treatment of cancer.<sup>4</sup> There are many secondary metabolites identified in plants such as *Drosera indica*, *Dionaea muscipula*, *Darlingtonia* and *Sarracenia* which possess anti-cancer property. Metabolites like naphthoquinones, phenolic acids and flavonoids are present in these insectivorous plants. Quercetin helps in cell cycle arrest and induces cell death in cervical cancer cells by mitochondrial apoptosis. Chlorogenic acid has the property of killing lung cancer cells. Vanillin helps in apoptosis of cervical cancer cells. Caffeic acid serves as an anti-inflammatory agent. Ellagic acid shows apoptosis of pancreatic cancer cells due to release of cytochrome c and activation of caspase. Quercetin is having anti-bacterial activity.<sup>13</sup>

## 2) Anti-diabetic activity

Earlier people use plants to treat the symptoms and causes of many diseases, and traditionally prepare most of their plant extracts as decoctions (boiling water extracts). In 2003, a number of Cree First Nations of Eeyou Istchee as well as the Cree Health Board initiated a research project in collaboration with six university laboratories, named the CIHR Team in Aboriginal Antidiabetic Medicines (CIHR-TAAM), to evaluate the safety and efficacy of culturally relevant medicines used to treat symptoms of Type 2 diabetes.<sup>14</sup>

The CIHR Team in Aboriginal Antidiabetic Medicines is a multidisciplinary team aimed at alleviating the impact of this disease by using a culturally adapted

approach: identification of medicinal plants with potential antidiabetic properties stemming from the Cree traditional pharmacopeia. Based on 15 symptoms of type 2 diabetes, team interviewed Cree elders and healers to identify several potential antidiabetic plants, 17 of which were found to be most promising. These plants were ranked according to their syndromic importance value for the treatment of diabetes using quantitative ethnobotanical methods.<sup>15</sup> *Sarracenia purpurea* L., known as the purple pitcher plant, was among the top ranked species. *S. purpurea* extract exhibited interesting antidiabetic potential in an initial screening study. In particular, *S. purpurea* extract principally increased glucose uptake in C<sub>2</sub>C<sub>12</sub> mouse muscle cells under basal and insulin stimulated conditions. This plant extract also decreased hepatic glucose output by reducing the activity of glucose-6-phosphatase, a key enzyme in gluconeogenesis and by increasing glucose storage through an increase in glycogen synthase activity. In addition, the extract also protected PC12 neuronal cells against cell death caused by hyper- or hypo glycaemic conditions. So, this plant exhibits both primary (glycemia-lowering) and secondary (protection against diabetes complications) antidiabetic activities.<sup>16</sup> It was shown here that the 80% ethanol extract generally results in significantly higher quantities of phenolics present, as well as the triterpenes. The only exceptions were the two most polar substances present (+)-catechin and morroniside.<sup>17</sup> This result was not surprising as higher quantities of phenolics have been consistently seen by other groups in alcohol extracts. In fact, this is one of the reasons why alcohol extracts are preferred in bioassays since relevant biological activities, such as antioxidant activity, are easier to observe in extracts with a greater phenolic concentration. Alcohols, being less polar solvents, are able to break down plant cell walls more efficiently and hence extract the necessary components. However, pure alcohol extracts are less efficient than alcohol/water mixtures. The 80% ethanol/water mixture normally used for extraction was optimized previously in the laboratory for phenolic extraction. Also, it is understandable that (+)-catechin and morroniside were present in significantly higher quantities in the water extract. They are the two most polar marker substances examined, as can be seen by their early retention time and logP values, and hence more readily extracted in the more polar solvent.<sup>18</sup>

## 3) Anti-herpes virus activity

Plants such as *Sarracenia purpurea* (*S. purpurea*), *Melissa officinalis*, *Clinacanthus nutans*, *Glycyrrhiza glabra*, *Rhus chinensis*, *Rhus javanica*, and *Punica granatum* have been reported to contain anti-herpetic activity. These herbs may possess important anti-herpetic compounds to treat recurrent HSV-1 infection. *S. purpurea* have the ability to inhibit the replication of poxviruses by inhibiting viral transcription. This study also explains that *S. purpurea* extracts have broad antiviral activity and inhibit the replication of HSV-1.<sup>19</sup>

Limited clinical trials for HSV-1 infection, performed by three different research groups, determined that a topical application of *S. purpurea*, provided rapid relief from the pain and improved healing of the viral associated lesions, as compared to the placebo group. These results support that *S. purpurea* may have bioactive anti-herpes components which may effectively treat recurrent HSV-1 symptoms. In the current study, concluded that *S. purpurea* extracts can inhibit the replication of HSV-1 through two different mechanisms of action.<sup>20</sup> The extracts directly inhibit extracellular virions or viral attachment to the host cell as well as inhibiting the expression of ICP4, ICP8 and gC when added at various times after infection. These results support the broader anti-viral activity of *S. purpurea* extracts against one pox and also herpes viruses. No cell toxicity was observed with *S. purpurea* extracts at the doses used (up to 120 µg/ml). Pitcher plant extract contains bioactive anti-herpes active constituents with limited or no cell toxicity at the doses

tested.<sup>21</sup> Pitcher plant extracts against poxviruses demonstrated that the extract did not disrupt the poxvirus envelope, here suggest that the extract is likely blocking HSV-1 attachment to the cell, although further studies to confirm this are required. The results presented also support that the *S. purpurea* extract inhibited replication of HSV-1 at a stage following viral uptake into the host cell. When the extract was added to viral infected cells up to 6 h.p.i, viral replication was inhibited. (Figure 4) *S. purpurea* has earlier been shown to inhibit poxvirus replication by inhibiting viral gene transcription.<sup>3</sup> Treatment with the extract at various stages during HSV-1 virus replication cycle resulted in a decreased manner in viral gene expression and a corresponding reduction in viral protein levels. Addition of the extract at different times post-infection suggests that the extract can inhibit gene expression. These results may suggest a common target between poxvirus and HSV-1 viral gene expression which is being inhibited by this extract.<sup>22,23</sup>

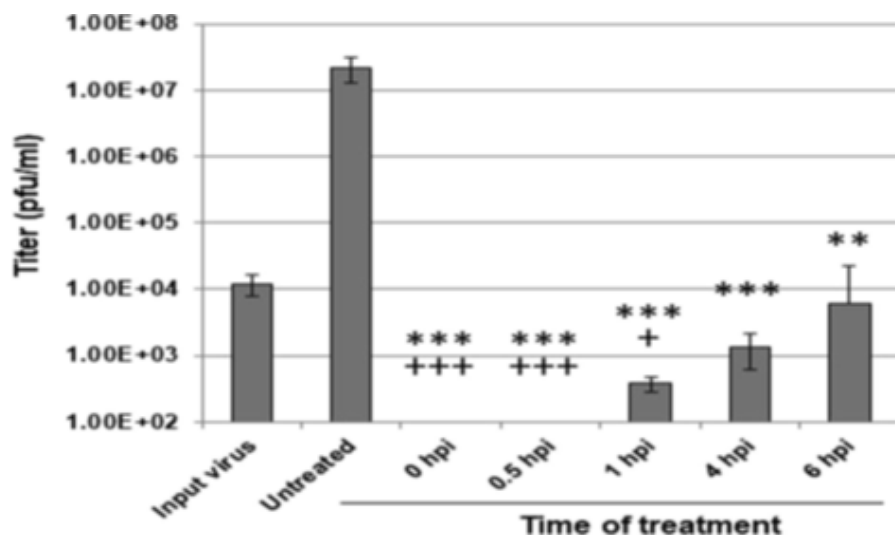


Figure 4: *S. purpurea* temporal inhibition of HSV-1 replication.<sup>3</sup>

#### 4) Anti-mycobacterial activity

People of Eastern Canada have used *S. purpurea* as a remedy for tuberculosis-like symptoms either by infusing the plant as a tea or through direct consumption of the plant. Bioassays have shown that methanolic extracts of *S. purpurea* inhibited the growth of *M. tuberculosis* H37Ra and these observations, in conjunction with the plants historical use by Canadian First Nations, have prompted research to identify and isolate the antimycobacterial constituents from *S. purpurea*.<sup>24</sup> Betulinaldehyde, Betulinic acid, and Ursolic acid were identified as the main constituents having antimycobacterial activity of *S. purpurea*. Antimycobacterial testing was performed using the culture broth supplied in Mycobacteria Growth Indicator Tubes in non-tissue culture treated, low binding, black 96-well microtitre plates sealed with polyester films (50 µm). The *S. purpurea* extract exhibited antimycobacterial activity at 100 µg/mL

against *M. tuberculosis* H37Ra in this screening bioassay and fractionation of the extract was bioassay guided using the MRA. Extracts of *S. purpurea* in methanol were therefore screened for antimycobacterial activity against *M. tuberculosis* H37Ra using the microplate resazurin assay. Bioassay guided fractionation involving solvent partition, flash chromatography, and normal phase HPLC led to the isolation of betulinaldehyde (0.03% dry weight), betulinic acid (0.08% dry weight), and Ursolic acid (0.02% dry weight) as the main antimycobacterial constituents. β-Sitosterol (0.02% dry weight) was also isolated, but was found to be significantly less active than the other triterpenes.<sup>24,25</sup> Each and every compound exhibited inhibitory activity against *M. tuberculosis* H37Ra with Betulinaldehyde and Ursolic acid being the most active (97.8 and 92.7 µM respectively) and β-sitosterol having only antimycobacterial activity (>1000 µM). Betulinic acid and Ursolic acid have been earlier

isolated from Pitcher plant. Every compound has been reported to exhibit antimycobacterial activity at levels similar to those observed for the *S. purpurea* natural products.<sup>26</sup>

### 5) Cytoprotective activity

The toxicities of *Sarracenia. purpurea* leaf as well as root extracts were established following 96 h exposure to cells in serum-free, normal glucose (11 mM) conditions. IC<sub>50</sub> concentrations were defined as the extract concentration eliciting a 50% loss of viable cells as compare to control cultures. Cell viability was determined by mitochondrial dehydrogenase cleavage of the formazan dye WST as compared to vehicle-treated (0.1% DMSO) control cells.<sup>2</sup> Both plant extracts were well-tolerated by cells but, with IC<sub>50</sub> concentrations of 129 µg/mL and 56 µg/mL, respectively, the leaf extract was less toxic than that of the root extract. PC12-AC cells can be differentiated to a peripheral catecholaminergic neuron phenotype by the combination of serum deprivation and treatment with nerve growth factor.<sup>27</sup> As such, these cells have commonly been used to model neuronal stress and serve as an accessible model of diabetic peripheral neuropathy repeatedly used by and others. Consistent with previous reports using the current protocol, the viability of vehicle-treated PC12-AC cells exposed to elevated glucose concentrations (150 mM) for 96 h was reduced by 40-50% relative to vehicle-treated cells under normal glucose conditions. This cell loss is glucose-specific and not due to osmotic stress as substitution of D-glucose for L-glucose abolishes toxicity.<sup>28</sup> Other studies have demonstrated a similar loss (30%) of PC12 cells exposed to 75 mM high glucose media once differentiated to aneuronal phenotype. To ascertain whether the protective activity of *S. purpurea* in the glucotoxicity model is organ-specific, root and leaf extracts were evaluated at various concentrations below their respective IC<sub>50</sub> concentrations (0-30 µg/mL for root and 0-100 µg/mL for leaf). The leaf extract reduced glucose-induced cell loss in a concentration-dependent manner up to 30 µg/mL but failed to provide protection at 100 µg/mL. Conversely, the root had no appreciable effect on glucose toxicity at low concentrations but significantly exacerbated cell loss when concentrations approached the IC<sub>50</sub> value. With the previously known bioactivities ascribed to quercetin and its glycosides, the isolation of hyperoside as one of the active metabolites responsible for *S. Purpurea*'s cytoprotective effects is not surprising. Experimental Data are consistent with previous results indicating that quercetin derivatives display anti-diabetic and neuroprotective activities relevant to the treatment or prevention of diabetic neuropathy. Major factors contributing to microvascular complications of diabetes, like neuropathy, include oxidative stress, the formation of advanced glycation end products (AGEs), and increased flux through the polyol pathway. As established anti-oxidants that inhibit both AGE formation and aldose reductase activity, quercetin derivatives potentially act through a number

of mechanisms. Although this study was not conducted in primary neurons, previously it was confirmed that the neuroprotective activity of plant compounds identified through preliminary testing in PC12 cells and recent studies have validated this finding in neuronally differentiated PC12 cultures. Moreover, since quercetin and quercetin glycosides prevent neuronal death in several in vitro and in vivo models of neurodegeneration, their activity in the current model is promising. The observed effects of the leaf extract are, however, of increased interest considering that SarapinW, a root extract of *S. purpurea*, is used to relieve pain. Though clinical evidence supporting SarapinW is incomplete and the putative active constituents remain unidentified, the preparation has been used for a variety of pain-related ailments. With further study, *S. purpurea* products could potentially provide both symptomatic relief and slowed progression of diabetic neuropathy through the preparation of two separate medicines, a leaf tincture and an alkaline root extract.<sup>29</sup>

### 6) Local Analgesic Effects

The marketed aqueous extract solution of *S. purpurea* (P-Bloc) was unable to modify the hoof withdrawal reflex latency (HWRL). Similar results were reported previously, using a different commercial extract of *S. purpurea* (Sarapin).<sup>30</sup> Furthermore, the lack of effect of a 1% ammonium sulfate solution in this model suggests that the anecdotally related analgesic effects of the aqueous extract of *S. purpurea* are unlikely to be mediated by the proposed neurolytical action of ammonium. The abaxial block model, that is measures HWRL, has been mostly used in veterinary medicine. The model represents a practical experimental approach with which to evaluate molecules with potential local anesthetic effects because the anatomical locations of the digital nerves at the metacarpus or metatarsusphalangeal joint are superficial and easily approached. Gerardo J. *et.al* found that the HWRL in their experiments was slightly shorter than that reported by other authors, so a result that could be explained by the increase in the heat energy provided by the lamp bulb in our custom-made heat projector lamp. However, the positive control using 2% lidocaine solution clearly showed that the HWRL was long enough to evaluate the local anesthetic effect of P-Bloc and 1% ammonium sulfate solution.<sup>31</sup> The commercially available aqueous extract solution of *S. purpurea* (P-Bloc) and the 1% ammonium sulfate solution did not modify the HWRL at early time points (6 hours) and up to 24 hours. On the other hand, the HWRL approach was able to detect the local anesthetic effect of a 2% lidocaine solution during the first 6 hours. Therefore, the possibility still exists that P-Bloc and the 1% ammonium sulfate solution could exert analgesia through a nonlocal anesthetic mechanism of action in a prolonged time frame, warranting further investigation.<sup>32,33</sup>

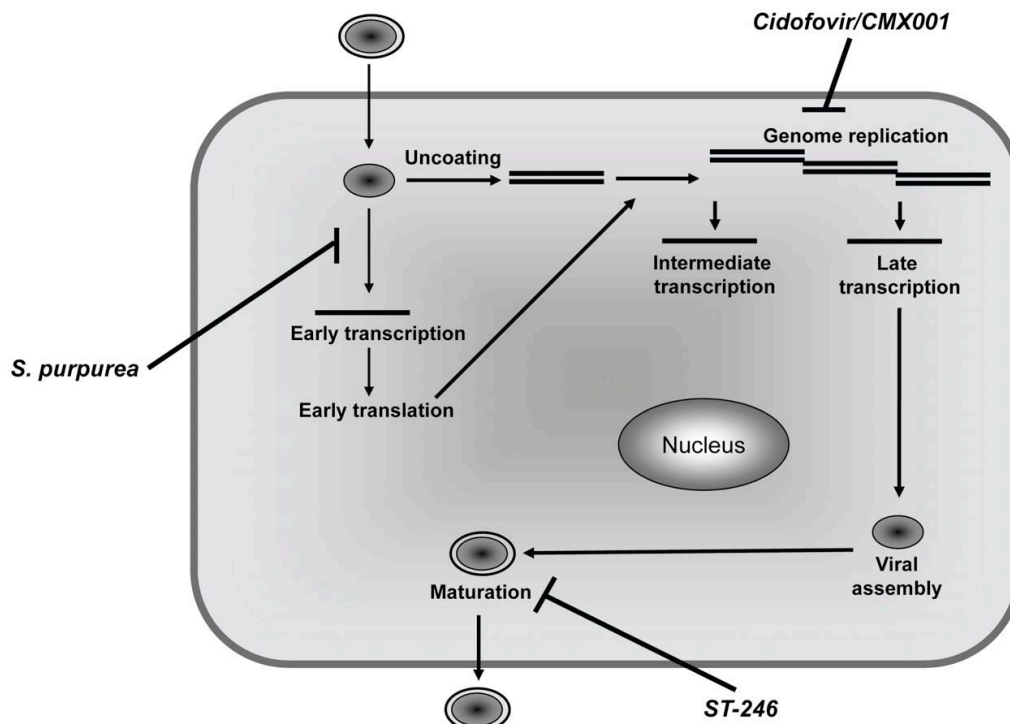
### 7) Anti-viral activity

Cidofovir and Purple pitcher plant are acting on different targets in the VACV (Vaccinia virus, smallpox



like virus) replication cycle and that Pitcher plant may be inhibiting virus replication at early stage in the replication cycle before to the induction of CPE.<sup>34</sup> To understand the mechanism of action for antiviral activity associated with Pitcher plant, various treatment schedules were tested. In cells which are treated with a single dose of *S. purpurea* overnight prior to infection with VACV followed by washing the cells and no inhibition of VACV replication was observed, suggesting the extract does not induce a cellular antiviral component.<sup>35</sup> Additionally, treating a purified

VACV stock with *S. purpurea* did not affect replication of the virus, which is indicating that the extract does not have a direct effect on free virus particles. To determine when *S. purpurea* treatment was most effective at preventing VACV replication, here examined the ability of *S. purpurea* to prevent VACV induced CPE when it is added at various times post-infection. In this test, cells were infected with VACV and after that they treated with *S. purpurea* at the indicated times post-infection.<sup>36,37</sup>



**Figure 5: Mechanism of action of poxvirus therapeutics. Illustration indicates the general replication cycle of VACV. The previously shown targets of known antipoxvirus compounds, cidofovir and ST-246, are shown, as well as the presumptive target of the *S. purpurea* extract. doi: 10.1371/journal.pone.0032610.g005**

**CONCLUSION:**

The World Health Organization has estimated more than 80 % of the world’s population in developing countries depends primarily on herbal medicines for their basic healthcare needs. In recent years, ethnobotanical and traditional uses of herbal compounds, especially those of medicinal plant origin, have received much more attention because they are well known for their efficacy and they are mostly believed to be very much safe for human to use for different activities. It is best to use the traditional approach in the search for new molecules to manage a variety of diseases. The leaves of *S. purpurea* have been in use since times immemorial to treat the wide range of indications. It has been subjected to quite extensive phytochemical,

experimental and clinical investigations. Experimental studies have demonstrated its Anti-diabetic activity, Anti-herpes virus activity, Anti-mycobacterial activity, Cytoprotective activity, Local analgesic effects and Anti-viral activity. Anti-diabetic activity, Anti-herpes virus activity, Anti-mycobacterial activity, Cytoprotective activity, Local analgesic effects and Anti-viral activity. (Table 2) A thorough review of the published literature on *S. purpurea* shows that it is a popular remedy in a variety of ethnic groups, as well as Ayurvedic and traditional practitioners for the treatment of a range of ailments. Researchers are exploring the different therapeutic potential of this plant as it is likely to have better therapeutic properties than are currently known in the literature.



**Table 2: Summary of Pharmacological activities exerted by *S. purpurea***

Sr. No.	Pharmacological Activity	Responsible extract /active constituents	References
1.	Anti-cancer activity	Ellagic acid, Quercetin, Caffeic acid, Chlorogenic acid, Vanillin	1,4,12,13
2.	Anti-diabetic activity	Catechin, Morroniside	14, 15, 16, 17, 18
3.	Anti-herpes activity	<i>S. purpurea</i> extract	3, 19, 20, 21, 22, 23
4.	Anti-mycobacterial activity	Betulinaldehyde, Betulinic acid, Ursolic acid	24, 25, 26
5.	Cytoprotective activity	Leaf and root extract	27, 28, 29
6.	Local analgesic activity	Aqueous extract	30, 31, 32, 33
7.	Anti-viral activity	<i>S. purpurea</i> extract	34, 35, 36, 37

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