Commentary

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Research Journal of Plant Pathology

2020
Vol.3 No.2:2

DOI: 10.36648/plant-pathology.3.2.2

Cissampelos pareira's Tale from the Benevolent World of Medicinal Plants

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Citation: Sahal D, Sharma U (2020) *Cissampelos Pareira*'s Tale from the Benevolent World of Medicinal Plants. Res J Plant Pathol Vol.3 No.2: 2

Received: September 15, 2020; **Accepted:** September 29, 2020; **Published:** October 06, 2020

Description

India's ethos epitomized in an ancient hymn is in essence aspiration for peace in among other things like space, water, land, sky, air, body and mind also the wish to have peace in the world of plants and the medicines that come from them. Dominated by rationality, we should not be blind to the fact that many truths of life may have no apparent rationale. In this context, it has been a puzzle for a very long time as to why plants should synthesize molecules that provide magical cure for diseases of animals including man. More directly, why must *Cinchona* tree make Quinine against malaria [1-3] or why should the Yew tree make Paclitaxel against cancer [4-6]? Also herbal medicines have been responsible for saving mankind since the times much before modern medicine came into being [2]. In this vein, *Cissampelos pareira* **(Figure 1)** a dioecious, perennial, twinning and climbing medicinal shrub belonging to the family *Menispermaceae* has been known in folk lore to have therapeutic benefits for menstrual cramps, threatened miscarriage, to ease childbirth, to stop uterine hemorrhages after childbirth, postpartum pain, fibroid tumors, cough, abdominal pain, fever, inflammation, indigestion, dysentery, wound healing and skin disorders [7,8]. It has also been found efficacious as anti-snake venom and in the treatment of malaria [9]. It is its reputation as a cure for malaria which inspired us to do chemical profiling of this plant to find which of its molecules may contribute to its antimalarial traits [10].

Figure 1: *Cissampelos pareira*.

We grew the drug sensitive and the drug resistant strains of malaria parasite *in vitro* in human red blood cells in wells of microtiter plates **(Figure 2)**. The absence of Nucleus in human red cells allowed us to use a DNA sensitive fluorescent dye *viz* SYBR Green to monitor the growth of malaria parasite in absence vs. presence of test samples coming from different parts (e.g. leaf, root, stem) of *Cissampelos pareira*.

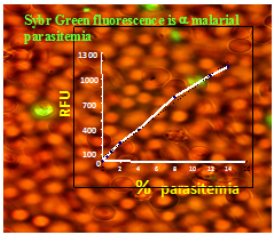

Figure 2: Mirotiter plate assay of antiplasmodial activity using SYBR Green fluorescence.

Before putting samples for testing, we extracted molecules from individual plant parts using different solvents like n-butanol, ethylacetate, methanol or water. Vacuum evaporation of solvents from solutions in each extract, allowed us to know the precise weights of each extract for quantitative estimation of the true antiplasmodial potency of each sample. As shown in **Figure 3**, a comparative analysis of relative potencies of extracts of different plant parts told us that it is the root of the plant that has the highest promise against malaria parasite.

Having identified root as a store house of antimalarial molecules, we went ahead with anti-malaria parasite activity guided isolation of molecules from the root extract. We used tools of chromatography and spectroscopy to separate molecules and identify their chemical structures. As shown in **Figure 4**, the root extract was a rich source of molecules with a wide range of chemical diversity. We correlated the potency of different fractions **(Figure 3)** with the chromatographic profiles **(Figure 4)**. It was apparent that anti malaria parasite activity was associated with some of the dominant peaks in the ethyl acetate fraction (**Figure 4**, panel C).

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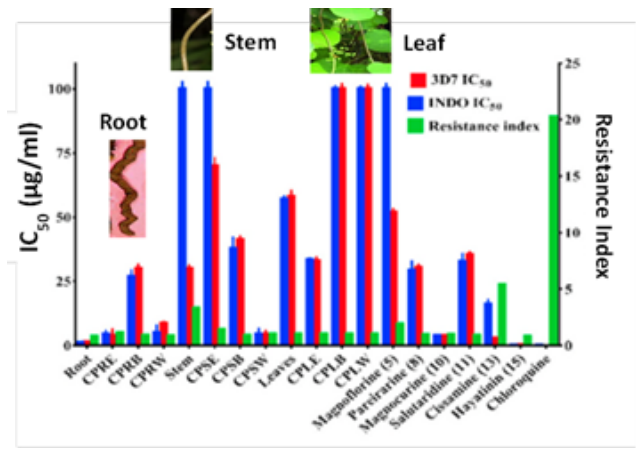

Figure 3: Efficacy of *Cissampelos pareira*(CP) plant part extracts and molecules purified therefrom against malaria parasite drug sensitive(3D7) and drug resistant(INDO) strains cultured in human red blood cells with growth of parasite monitored *via* SYBR Green fluorescence. IC_{50} is concentration of sample required to restrict growth of parasite to 50%; Resistance index (green bars) is ratio of IC_{50}(chloroquine Resistant strain) to IC_{50} (chloroquine sensitive strain). Note that unlike the very high resistance index for antimalarial drug Chloroquine, the corresponding green bars for plant samples are quite small. R, S, L: Root, Stem, Leaf; E,B,W: ethyl acetate, Butanol, water [11].

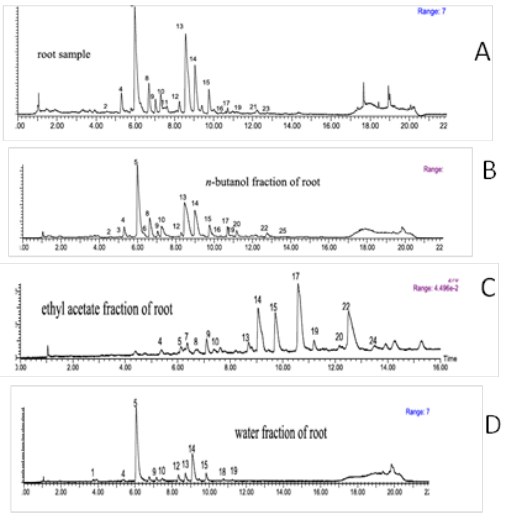

Figure 4: UPLC-DAD chromatograms of total root extract (A) and its different solvent extracts (B-D). Due to characteristic structural differences, different molecules are seen to concentrate in different solvents. Notice the intense signals in ethyl actetate fraction (B) at retention times where the same are considerably weak in the total extract (A). The identity of peaks marked 5,8,10,11,13 and 15 is given in Figure 3 [11].

We isolated each of the molecules and determined their potencies against the malaria parasite. Peak 15(**Figure 4**, panel C)identified as Hayatinine **(Figure 5)** was found to be the most potent (IC_{50} 0.4 µM) anti-malaria parasite molecule.

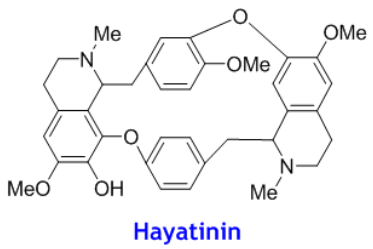

Figure 5: Chemical Structure of Hayatinin isolated from the root of *Cissampelos pareira* and found to be the most potent (IC_{50} 0.4 µM) against drug resistant malaria parasite.

It was a sense of joy to have isolated pure molecules from the crude extract of the root of *Cissampelos pareira* with promise against malaria. However its translation into a drug for curing a child suffering from malaria requires that we find out which other molecules in the crude extract could facilitate the drug action of Hayatinin and thwart the development of resistance against Hayatinin. It must also be remembered that the molecular bases of several other therapaeutic properties of *Cissampelos pareira* including its anti snake venom and wound healing activities remain to be determined. Our salute to the polypharmacy that *Cissampelos pareira* is!

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