

Lateral Flow Immunoassay as Novel Screening Tool for Potential Cross-Reacting Protein in Crude Plant Latex as Tested for Plasmodium, Dengue, HBV, HCV, HIV

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Abstract

In vitro diagnostic kits use immune chromatographic assay with gold nanoparticle conjugated antigen / antibody coated in the kit that specifically detects the antigen of the pathogen or antibody to a pathogen in the blood or serum tested. Such kits are available for wide variety of diseases and different kits could use different antigens targeted. In this research article I present the novel finding that when the latex of *Calotropis gigantea* or *Thevetia peruviana* is used as the sample in a kit targeting the Histidine rich protein of *Plasmodium falciparum*, the kit displayed positive reaction which indicates homologous protein exists in the latex of these two plants to that of the Histidine rich protein of *Plasmodium falciparum*. Similarly, it was noticed for the LDH protein of *P. vivax* and *Calotropis gigantea*. Further it was noticed that the latex of *Calotropis gigantea*, *Tabernaemontana divaricate* had activity against HCV, Dengue, HIV. The latex of *Carica Papaya* had activity against HCV. Hence this article brings out the novel concept of use of human pathogen specific lateral flow kits for screening of plant latex and sap to identify potential cross-reacting proteins that even a layman can screen and doesn't require expertise like docking studies. Further this could pave way for layman to use these LFA for screening of plant proteins of medicinal interest or to use of these plant extracts for detecting antigens, antibodies in human sera of a particular pathogen that can cross react with plant latex and produce agglutination.

Keywords: Drug Discovery, Plasmodium, HCV, HIV, Dengue, Lateral Flow

Introduction

Many plant species have been reported in literature to have anti-microbial effects where in their solvent extracts have shown to be effective *in vitro* or *in vivo*. These plants as such could be toxic or nontoxic in nature. *Calotropis* which is toxic to humans has metabolites that are anti-plasmodial (1), antiviral for Influenza (2) and anti-bacterial (3).

Similarly other plants of medical importance like *Tabernaemontana divaricate* have been shown to have anti-viral effects against Dengue. Other plants like *Thevetia* is known for its anti-oxidant properties (4). It

has also been shown that methanolic extracts of *Carica papaya* is useful for its anti-viral properties against HCV, HIV (5). It has been shown that medicinal plant product-based inhibition of HCV replication is possible (6).

Plants have also been used as models for generation of plant antibodies for human and animal diseases in which recombinant protein is expressed in plant such as tobacco, potato, alfalfa (7). It has also been reported in literature of strange cross-reacting protein between tobacco mosaic virus and a fruit protein thaumatin (8). Hence it might be interesting and valuable to screen crude plant extracts for cross reacting proteins with human and animal viruses for potential drug search. Molecular mimicry resulting in antibodies against tobacco mosaic virus is known to exist in humans (9). Towards this, people have used computational biology tools, drug docking studies (10). But docking studies requires expertise and is not possible for everyone to perform that without extensive knowledge and training. Knowledge databases like IMPPAT - (A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics) available at <https://cb.imsc.res.in/imppat> has information on medicinal plants and phytochemicals from plants that could act against diseases.

This article brings out a simple lateral flow cross reactivity-based tool to screen for potential drugs in crude plant extract. Normally lateral flows are used for specific diagnosis of human, animal or plant diseases (11). The lateral flow assay works on capillary action of the tested solution that contains the analyte and is dropped at a point called the sample point of the lateral flow device. The sample solution with the analyte moves forward to the conjugate pad that has the gold nanoparticle tagged antigen or antibody which binds to sample analyte and moves further to coated zones of specific diagnostic antigen or antibody that produces a colored band at the test site and control site of the lateral flow device (12).

Materials and Methods

1. Lateral flow assay: Rapid diagnostic kits were purchased for *Plasmodium* (Abbott) and for Dengue, HCV, HIV, HBV (Med source). The plant latex or sap were dropped at sample points of the lateral flow assay and the results were noted within 1-10' as soon as the control line turned positive. In case of *Plasmodium* LFA of Abbott, the test line coat is antibody against the histidine rich protein of *P. falciparum* and antibody against LDH of *P. vivax*. In case of HCV, of Med source, the test line has recombinant antigens of HCV for detection of anti HCV antibodies and similarly for HIV, the test line coated with antigens for detection of anti-HIV antibodies. In case of Dengue LFA dual- IgM and NS1 Ag was used.
2. Bioinformatic analysis: The phytochemical compound identified through IMPPAT database (<https://cb.imsc.res.in/imppat>) for a particular medicinal plant was searched for interacting protein partners for the particular pathogen by STITCH database (<http://stitch.embl.de/>)

Results

The results have been tabulated in Table 1. The crude latex of *Calotropis gigantea*, *Thevetia peruviana* had cross reactivity for the Histidine rich protein of *Plasmodium falciparum* in the lateral flow assay. Further *Calotropis* latex too had cross reactivity for lactate dehydrogenase of *P. vivax*. Further it was noticed that the latex of *Calotropis*, *Tabernaemontana divaricate*, *Carica papaya* had cross-reactivity against HCV in terms of reacting with recombinant HCV antigens that were meant for detection of anti-HCV antibody. The latex of *Calotropis*, *Tabernaemontana divaricate* was also able to

cross react with Dengue NS1 antigen and with the recombinant antigens of HIV meant to capture anti-HIV antibodies.

Further to elucidate if the available phytochemicals in the plant of interest as listed in the IMPPAT database has any interacting protein network, STITCH database was used.

Medicinally important compounds detected through this database were camptothecin in *Tabernaemontana*. Figure 1 displays the 3-D structure of it and its interacting protein partners. Medicinally important compounds like quercetin, kaempferol detected in *Thevetia* were analysed for interacting proteins as in Figure 2,3.

Discussion

Although use of rapid lateral flow kit is not new for disease diagnosis (10), this article brings out the novel possibility of using them for drug screening in crude extracts of plant which has not been reported. The use of lateral flow does not require expertise like that of molecular docking studies. Although nine plants were screened, it can be seen that certain plants had sensitivity for few pathogens and not all as indicated in Table 1 of the results. This indicates that the plant latex has proteins that can cross react with the coated pathogen specific antigen or antibody.

Our study indicated for the first time that the latex of *Thevetia peruviana* had reactivity for *Plasmodium falciparum*, Dengue, HCV. The latex of *Calotropis* had reactivity for *Plasmodium vivax*, HCV, HBV, HIV. *Tabernaemontana divaricate* had reactivity for Dengue, HCV, HIV and *Caricacapyah* had reactivity for only HCV. This is novel since if there is specific interaction of coated antigen, antibody of human infectious agents with that of plant crude latex, then it could be used as a method to screen plants at field sites and even by a layman to find potential sources of new medicines.

Literature has shown that medicinal plant (*Tabernaemontana divaricate*) extract is effective against dengue infection in animal models (13). It has been shown that toxic plants have been used for viral infections (14). Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) database has information on all the possible phytochemicals and plants that can act as anti-microbials. The IMPPAT database indicates the presence of compounds like Quercetin in *Thevetia* and camptothecin in *Tabernaemontana* which are known to be anti virals (15, 16,17,18,19) and the use of this database is described in literature (20). As shown in Figure 1,2,3 these compounds have many interacting protein partners and these proteins could have cross-reacted with that of the coated antigen / antibody in the lateral flow assay producing a cross-reactivity.

Hence although it is well established that medicinal plants have anti-microbial compounds, the methods to detect it, requires technical expertise and expensive equipment. But this article brings out the novel possibility of use of human disease detection lateral flow to detect cross-reacting plant proteins.

Further detection of this cross-reactivity could also pave way for a cheap plant extract – human sera agglutination assay that can be useful for detection of specific pathogens.

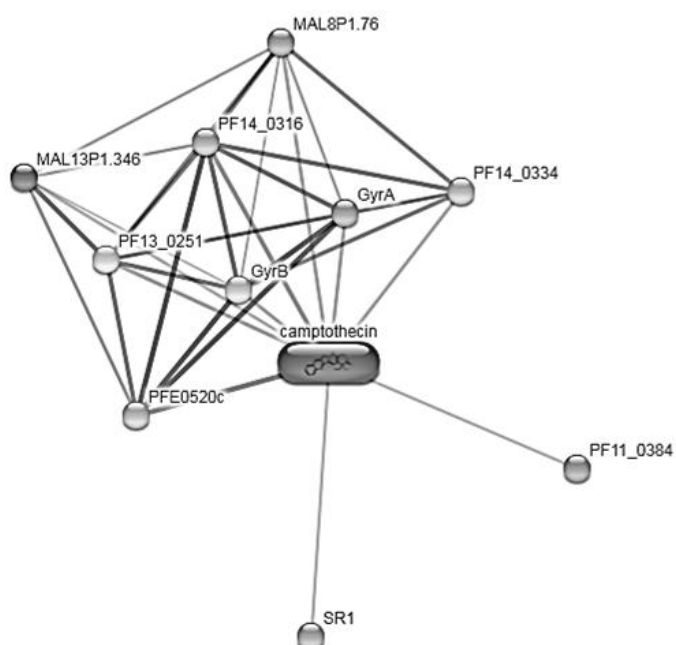
Conclusion

Rapid lateral flow kits could be used in field testing of cross-reacting plant proteins to human pathogens. Plant sap or latex does have specific cross reactivity to different human pathogens.

Plant extract tested			Tested with the Commercial kit for cross reactivity						
			Abbott		Med source				
			<i>P.falciparum</i>	<i>P.vivax</i>	Dengue	Dengue	HC V	HB S	HI V
			Antibody to Histidine rich protein	Antibody to Lactose dehydrogenase	Antibody to NS1	NS1 Antigen	HC V Antigen	HB S	HI V Ag
1	<i>Thevetia peruviana</i> <i>Cascabellathevetia</i>	Reactivity to Latex	+	-	+	-	+	-	-
2	<i>Calotropis gigantea</i>	Reactivity to Latex	+	+	-	-	+	+	+
3	<i>Carica papaya</i>	Reactivity to Latex	-	-	-	-	+	-	-
4	Aloe vera	Reactivity to plant sap	-	-					
5	<i>Moringa oleifera</i>	Reactivity to plant sap	-	-					
6	<i>Tabernaemontanadivariata</i>	Reactivity	-	-	+	+	+		+

		to Latex							
7	<i>Codiaeum variegatum</i>	Reactivity to plant sap	-	-					
8	<i>Nerium oleandrum</i>	Reactivity to latex	-	-					
9	<i>Artocarpus heterophyllus</i>	Reactivity to latex	-	-	-	-	-	-	-

Table 1. Cross reactivity to different plant latex / sap



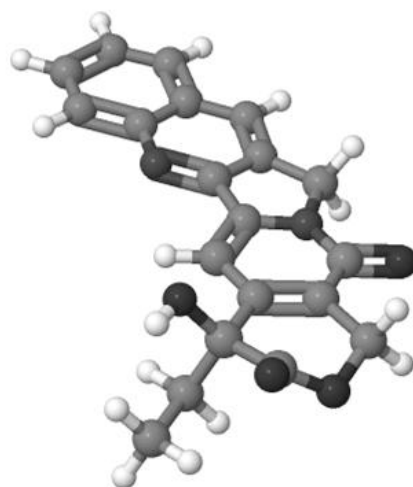


Figure 1 Camptothecin and its interacting protein in *Plasmodium falciparum*

(Left) The structure of camptothecin as simulated in JSMOL is given below.

The interacting proteins of this compound with that of *Plasmodium falciparum* as analyzed by STITCH database is shown in the right-side image.

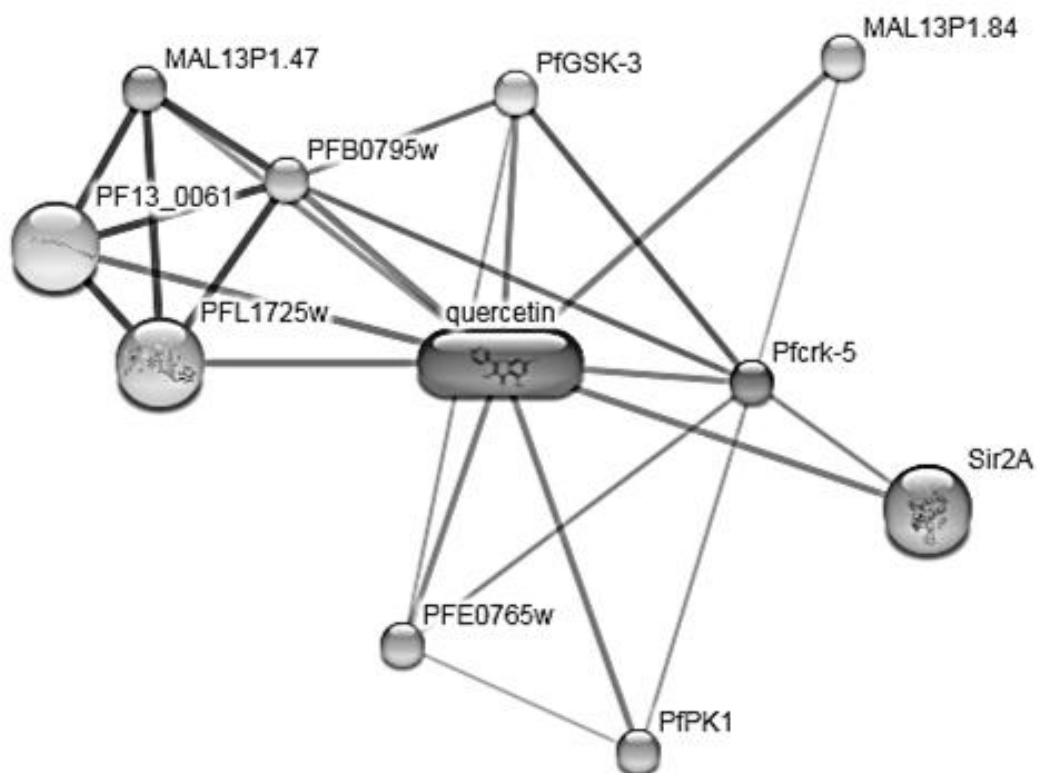


Figure 2 Interacting protein partners of Quercetin in *Plasmodium falciparum*

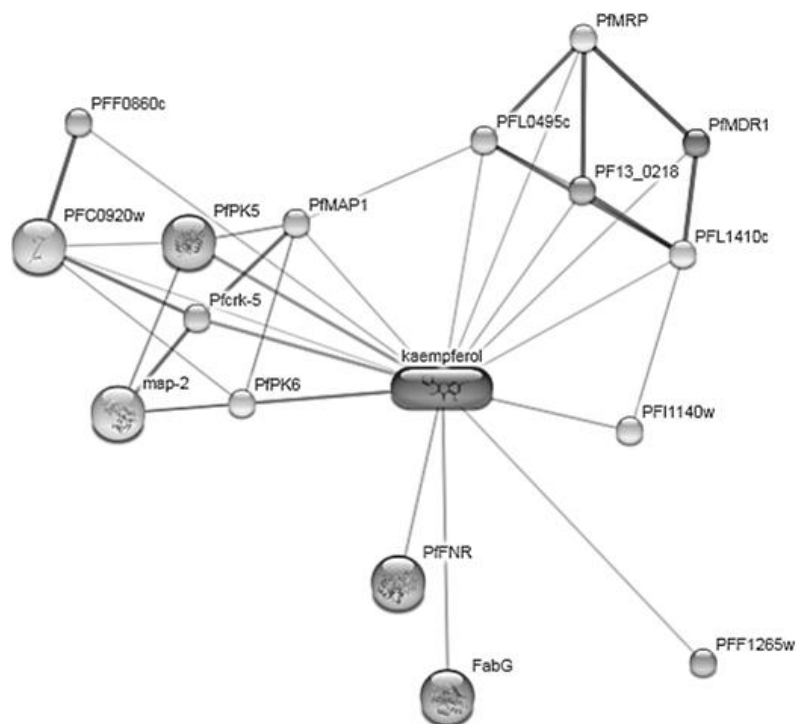


Figure 3 Kaempferol and its interacting protein partners

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