Anti-plasmodial potential of crude alkaloidal extract of three plants used in traditional medicine in India

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Abstract: Malaria is one of the most important tropical diseases and the greatest cause of morbidity and mortality in India. The search for new antimalarial compounds has been necessitated by Plasmodium falciparum resistance to standard antimalarial drugs. Plants are important source of biologically active compounds and have potential for the development of novel antimalarial drugs. Since a number of alkaloids have been successfully used for the treatment of malaria since ancient time, in this study the crude alkaloidal extract of three young plants Carica papaya Linn. (Family: Caricaceae), Datura innoxia Mill. (Family: Solanaceae) and Ricinus communis Linn (Euphorbiaceae) were evaluated against Plasmodium falciparum 3D7. The mean inhibitory concentration (IC50), the mean cytotoxic concentration and the selectivity index were estimated. The cytotoxicity was estimated on Madin-Darby Canine Kidney (MDCK) cell line in maximum dose tested. The alkaloidal extract of C. papaya showed stronger antiplasmodial activity as compared to D. innoxia and R. communis, the IC50 values in the range of 28.35 to 93.17μg/mL. Results demonstrated alkaloids as the putative active compound showing promising antimalarial effect.

Key words: Carica papaya, Datura innoxia, Ricinus communis, Plasmodium falciparum, antimalarial

I. Introduction

Malaria represents world’s greatest public health problem in terms of number of people affected, levels of morbidity and mortality. About 3.4 billions worldwide are exposed annually, with 1.2 billion at high risk [1]. The alarming rate at which Plasmodium falciparum has developed resistance to chloroquine and other synthetic antimalarial drugs makes it necessary to search for more effective antimalarial compounds [2]. Developing countries, where malaria is endemic, depend strongly on traditional medicine as a source for inexpensive treatment of this disease [3]. However, scientific data to validate the antimalarial properties of these herbal remedies are scarce.

Alkaloids are one of the most important classes of natural products providing drugs since ancient times [4]. A number of alkaloids have been successfully used for the treatment of parasitic infection. Quinoline based antimalarials which include alkaloids consist of quinine from Cinchona and its derivatives are the most commonly used drugs against malaria [5, 6]. In the present study an attempt has been made to evaluate the crude alkaloidal extract of Carica papaya, Datura innoxia and Ricinus communis against Plasmodium falciparum 3D7 by in vitro assay.

Several species of Caricaceae have been used as remedy against a variety of diseases [7]. Papaya is a perennial plant, and it is presently distributed over the whole tropical area. The leaves of papaya have been shown to contain many active components and the extract of the leaf has been used for various disorders including cancer and infectious diseases. Many scientific investigations have been conducted to evaluate the biological activities of various parts of C. papaya, including fruits shoots, leaves; rind seeds roots and latex. The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level [8].

Datura innoxia Mill. (Solanaceae) is the wide spread species of the genus Datura and is well known for its use in traditional Indian medicine for centuries [9]. There are many different species in the Datura genus. It is commonly known as thorn apple belonging to the family Solanaceae. The phytoconstituents such as flavonoids, phenols, terpenes are found in Datura and the main constituents include alkaloids [10]. It is one of the most important medicinal herbs used worldwide due to its anti-inflammatory property [11, 12]. Several scientific studies and the results of anti-microbial, antioxidants and phytochemical screening of crude extract of this plant have been reported earlier [13]. Ricinus communis Linn (Euphorbiaceae) another plant selected for this study is widely distributed in India and reported to possess hepatoprotective, antidiabetic and anti-inflammatory activities [14, 15, 16].

A number of alkaloids have been successfully used for the treatment of malaria since ancient time. The present study aims to evaluate antiplasmodial potential of crude alkaloidal extract from three different plants against chloroquine sensitive Plasmodium falciparum 3D7.
II. Materials and Methods

4.1. Collection of plant materials
Leaves and aerial parts of all three plants were collected from Mumbai, (18° 55´ N, 72° 54´ E) India during June – September, 2013.

4.1.5. Identification of plants
Plants were identified by Dr. U. C. Bapat, Director, Blatter Herbarium, Department of Botany, St. Xavier’s College, Mumbai. Voucher specimens of all three plants Carica papaya L. (accession No. 14830) and Datura innoxia Mill. (accession No. U.P. 760) and Ricinus communis L. (accession no, 117) have been deposited for future reference.

4.1.6. Extraction procedure for crude alkaloids
Different plant parts were air dried powdered and processed for alkaloid extraction using standard protocol [17, 18]. Briefly, powdered plant material (10 g) was moistened with 5 mL of NH4OH (25%, m/m) and extracted with methanol for two days at room temperature. The extract was filtered and the solvent was evaporated in a rotary evaporator under reduced pressure at 40°C. The residue was dissolved in 2% H2SO4 in distilled water, filtered and extracted with petroleum ether to remove fat material. After basifying the aqueous solution to pH 9-10 with NH4OH (25%, m/m), it was extracted with chloroform, partitioned with distilled water to neutral pH, concentrated to dryness under reduced pressure to obtain crude alkaloids.

4.1.7. Parasite cultivation
The Plasmodium falciparum 3D7 strain was procured from Indian Institute of Technology (IIT), Mumbai, India, was maintained in continuous culture by the modified method of Jensen and Trager (1980) in O+ human red blood cells at a 5% haematocrit in RPMI 1640 medium, supplemented with L-glutamine (4.2mM), HEPES (25 mM), NaHCO3 (25 mM) hypoxanthine (6.8 M), 0.5% AlbumaxII (Invitrogen) and 50µg/ml Gentamicin [19]. Cultures were incubated at 37°C in an atmosphere of 5% CO2, 91% N2, and 3% O2. Parasite cultures were synchronized to ring stage by treatment with 5% D-sorbitol [20]. Chloroquine was used as positive control while 0.5% DMSO and 0.1% methanol as solvent control.

4.1.8. In vitro antiplasmodial assay
The in vitro antimalarial evaluation was done according to WHO, 2001 guidelines [21]. The extracts were filter sterilized and different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) were incorporated in 96 well tissue culture plate with 1-2% parasitemia and 2% haematocrit. The plates were incubated at 37°C in CO2 incubator and parasitemia was evaluated after 48 h by light microscopy using Giemsa-stained smears as described by Le Bras and Deloron [22].

2.1.4 Cytotoxicity assay
The cytotoxic effects were determined with MDCK cells, using the MTT tetrazolium-based colorimetric technique [23]. The selectivity index (SI), corresponding to the ratio between cytotoxic concentration on MDCK cell line to the antiplasmodial activity, was calculated for each test extract according to the following formula:
Selectivity index (SI) = Ratio CC50/IC50

III. Results
The basic measurement of antimalarial activity used in this study was the reduction in number of parasitized erythrocytes in extract treated test cultures as compared to control (100% parasitemia) without drug at 48 h of incubation period. Results are presented in table-1, Figure-1).
From the literature antimalarial activity of extracts was defined according to the IC50 values obtained. An extract showing an IC50 value ≤ 50 µg/mL was classified as active and extracts with activity beyond this range were considered in active [24]. Based on this classification, the alkaloidal extract of Carica papaya L. induce a significant decrease of parasite proliferation showed promising antimalarial activity (IC50 of 28.35µg/mL) as compared to Datura innoxia (IC50 of 42.32 µg/mL), and Ricinus communis (IC50 of 98.52µg/mL). In the present investigation extracts were found to be non-cytotoxic on MDCK cell line in maximum dose tested in two plants, the SI was>1 in case of Carica papaya (3.62 ) and Datura innoxia ( 1.80).
Where as in case of R. communis [0.94] the extract was slightly toxic. The results are summarised in table 1.

IV. Discussion
Alkaloids are the most efficient therapeutically significant plant substances, pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents [25]. The remarkable activity of quinine and related...
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drugs and the success of artemisinin have stimulated the search for new plant-derived antimalarials. Different solvent extract of any particular plant hold different antimalarial activity. Most of the antimalarial studies reported so far against these plants used aqueous and organic solvent extracts of [26,27, 28]. The present study aims to investigate the antimalarial properties of crude alkaloidal extract of C. papaya, D. innoxia and R. communis against Plasmodium falciparum 3D7.

To estimate the potential of molecules or extracts to inhibit parasite growth without toxicity, the selectivity index (SI) was introduced. Low SI indicates that the antimalarial activity is probably due to cytotoxicity rather than activity against the parasite themselves. In contrast, high SI should offer the potential of safer therapy [29]. In the present investigation the SI was 1 in case of Carica papaya was (3.62) and Datura innoxia (1.80). This observation may be an indicator of their safety as drugs for mammalian organism. Where as in case of R. communis (0.94) the low SI indicates that the anti-plasmodial activity may be probably due to cytotoxicity rather than activity against the parasite themselves. Compounds responsible for the antimalarial effects are under investigation.

V. Conclusions

This study supports continued investigations of meditational plant as potential source of alternative antimalarial agents. Aerial parts of Carica papaya at early flowering stage had shown promising anti-plasmodial activity and low toxicity as compared to Datura innoxia and Ricinus communis. The results are encouraging and warrant further investigation of purified alkaloids in vivo in murine malaria model. The results are encouraging and further work is needed for complete isolation, identification, and characterization to elucidate the active compound and their in vivo antiplasmodial efficacy in murine malaria model.

Acknowledgement

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Table 1: In vitro antiplasmodial activity, cytotoxicity, and selectivity index of crude alkaloidal extract of plants against *Plasmodium falciparum* 3D7

<table>
<thead>
<tr>
<th>SN.</th>
<th>Plant</th>
<th>Alkaloid Extract</th>
<th>Antiplasmodial (IC50, μg/mL)</th>
<th>Cytotoxicity MDCK (CC50, μg/mL)</th>
<th>Selectivity index(SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>cacirca papaya</td>
<td>Aerial parts</td>
<td>28.35 ± 3.23</td>
<td>102.74 ± 3.1</td>
<td>3.62</td>
</tr>
<tr>
<td>2.</td>
<td>Daturainnoxia</td>
<td>Aerial parts</td>
<td>42.32 ± 8.01</td>
<td>76.13 ± 5.3</td>
<td>1.80</td>
</tr>
<tr>
<td>3.</td>
<td>Ricinuscommunis</td>
<td>leaves</td>
<td>98.52 ± 2.17</td>
<td>94.18 ± 1.6</td>
<td>0.94</td>
</tr>
</tbody>
</table>

IC50, the inhibitory concentration of extract that induced 50% reduction in parasitemia

CC50: the drug concentration that reduced the number of viable MDCK cells by 50%

Selectivity index (SI) = Ratio CC50/IC50

![Figure 1: Effect of three plant extract on percent inhibition of parasitemia](image-url)