



Asian Pacific Journal of Tropical Biomedicine

Journal homepage: www.apjtb.org



doi: 10.4103/2221–1691.225619

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Ethnobotanical database based screening and identification of potential plant species with antiplasmodial activity against chloroquine–sensitive (3D7) strain of *Plasmodium falciparum*

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ARTICLE INFO

Article history:

Received 15 November 2017

Revision 7 December 2017

Accepted 12 January 2018

Available online 16 February 2018

Keywords:

Antiplasmodial

Antioxidant

Plasmodium falciparum

Ethnobotany

ABSTRACT

Objective: To evaluate the antiplasmodial activity of aqueous-methanolic plant extracts of nine plant species selected, based on ethnobotanical data. **Methods:** Based on ethnobotanical database, the selected plants were tested for their antiplasmodial activity against chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*. Qualitative tests and high performance thin layer chromatography analysis were carried out to explore the phytochemicals present in the plant extracts. 1,1-diphenyl-2-picrylhydrazyl antioxidant activity was also determined to check the antioxidant activity of the plant extracts. **Results:** *Moringa oleifera* (IC₅₀: 3.906 µg/mL), *Acalypha indica* (IC₅₀: 3.906 µg/mL), *Hyptis suaveolens* (IC₅₀: 3.906 µg/mL), *Mangifera indica* (IC₅₀: 4.150 µg/mL) and *Averrhoa bilimbi* (IC₅₀: 4.881 µg/mL) showed very good antiplasmodial activity. **Conclusions:** Crude extracts of *Mangifera indica* and *Hyptis suaveolens* demonstrated the most efficacious antimalarial activity. A bioassay-guided fractionation of these extracts to identify the lead compound is proved to be useful. The results validate the traditional use of the selected plants as antimalarials.

1. Introduction

There are five main species of *Plasmodium*, namely *Plasmodium vivax*, *Plasmodium knowlesi*, *Plasmodium ovale* and *Plasmodium malariae* and *Plasmodium falciparum* (*P. falciparum*). Among them *P. falciparum* is the most widespread and is considered to be deadly since it causes fatal complications[1–4]. According to WHO report, in 2015, nearly 438 000 malaria deaths occurred worldwide due to *P. falciparum*[5]. One of the major obstacles in fighting malaria is “drug-resistance”. *Plasmodium* species have gained resistance to artemisinin, which was introduced in 1971 as an antimalarial

drug. The first case of resistance was reported in 1980[6]. Most antimalarial drugs like quinine and artemisinin were developed on the basis of serendipitous discovery of the antimalarial activity of natural products[7]. About 80% of the world’s population still rely on herbal drugs for their health care[8]. Since ancient ages, humans have been looking forward to nature to cater to their basic needs, especially medicines. Most of the potential drug leads are plant-based[9]. Natural products play a key role in the production of

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How to cite this article: Noronha M, Guleria S, Jani D, George LB, Highland H, Subramanian RB. Ethnobotanical database based screening and identification of potential plant species with antiplasmodial activity against chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*. Asian Pac J Trop Biomed 2018; 8(2): 92-97.

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bioactive molecules and pharmaceutical agents[10]. The first written records on utilization of plants in medicine date back to 2600 BC[11]. Plant metabolites can provide us with novel lead compounds which can be used as template to develop antimalarial drugs[12]. Bearing these points in mind, the plethora of phytochemical constituents present in plant extract can be used to test the potency against *P. falciparum*. Plants were randomly selected for antimalarial property, some decades ago. This approach seemed time consuming. An alternative approach is to select plants based on their ethnobotanical and ethnopharmacological information[13–16]. Therefore, the present work attempts to identify and characterize a novel antimalarial substance from plants, based on the traditional use of plants.

2. Material and methods

2.1. Plant collection

Based on ethnobotanical literature survey, plants which possessed antipyretic and antimalarial properties were targeted. Nine such plants were selected and authenticated by Dr. A.S. Reddy, P. G. Department of Biosciences, Sardar Patel University. The same were also checked at www.theplantlist.org

The plants parts of *Mangifera indica* (*M. indica*) L. belonging to the family Anacardiaceae, *Aegle marmelos* (*A. marmelos*) L. Correa belonging to the family Rutaceae, *Ficus religiosa* (*F. religiosa*) L. belonging to the family Moraceae and *Moringa oleifera* (*M. oleifera*) Lam. belonging to the family Moringaceae, were collected from the same location (GPS location 22°34'11.6"N 72°54'34.1"E). *Acalypha indica* (*A. indica*) L from the family Euphorbiaceae was collected from the location 22°34'14.0"N 72°54'42.0"E, *Hyptis suaveolens* L. Poit. from the family Lamiaceae was collected from the location 22°33'42.5"N 72°55'44.3"E. *Ficus racemosa* (*F. racemosa*) L. belonging to the family Moraceae and *Averrhoa bilimbi* (*A. bilimbi*) L. belonging to the family Oxalidaceae were collected from the location 22°34'16.8"N 72°54'41.9"E and 20°16'15.5"N 73°00'22.6"E, respectively. The voucher specimen number of the collected plant samples are as follows: *M. indica* (PGBRM01), *A. marmelos* (fruit) (PGBRM02), *F. religiosa* (PGBRM03), *A. indica* (PGBRM04), *M. oleifera* (PGBRM05), *H. suaveolens* (PGBRM06), *F. racemosa* (PGBRM07), *A. marmelos* (leaf) (PGBRM02) and *A. bilimbi* (PGBRM08).

2.2. Plant extraction

Fresh plants were washed under running tap water and kept for shade drying for 7–10 d. After drying, the plant parts were pulverized to fine powder and stored in air tight containers for further analysis. The plant powder was defatted using petroleum ether (60–80 °C) and kept at 37 °C under continuous shaking condition.

The marc was then extracted with distilled water: methanol (70:30) in soxhlet extractor at 50 °C till the colour turned colourless in the siphon. The extract was first concentrated using a rotary evaporator under reduced pressure of 22–26 mmHg at 50 °C. When kept at steady state, the concentrated extract deposited crystals. The yield was collected and stored at 4 °C[17].

2.3. Phytochemical screening

The hydroalcoholic extracts of the samples were screened for various phytochemical constituents, using standard methods[17]. They were analyzed for the presence of alkaloids, phenols, glycosides, flavonoids, tannins, saponins, steroids and triterpenoids.

2.4. High performance thin layer chromatography (HPTLC)

HPTLC is considered to be the best approach to separate phytocomponents[17]. The crude plant extracts were dissolved in hydroalcohol (70:30) and 7 µL quantity of sample was applied onto the HPTLC plate, silica gel 60F₂₅₄ using CAMAG Linomat 5 injector. Camag twin-trough chamber was formerly saturated with the mobile phase ethylacetate: methanol: water (81:11:8) for 30 min and then the plate was kept for development. Plates were scanned at 366 nm with tungsten lamp using Camag Scanner III in combination with winCATS III software. The chromatographic finger print was developed for the detection of phytoconstituents present in the plant extracts and their R_f values were tabularized.

2.5. In-vitro percentage inhibition of entry of parasites

Chloroquine-sensitive (3D7), the chloroquine-sensitive strain of *P. falciparum*, obtained from NIMR, New Delhi, was used to test the antimalarial efficacy of the nine plant extracts. The *in-vitro* percentage inhibition of entry of parasites was determined by the method used by Jonville *et al*, with slight modification[18]. Assays were performed in sterile 96-well microtiter plates. Normal RBCs were treated with the plant extracts and exposed to infected RBCs with 2% parasitaemia for 24 h in CO₂ incubator. Normal RBCs without any kind of treatment were considered as control. The counting of number of schizonts in the control and treated samples was performed by making thin smear and staining them with JSB-I and JSB-II. The results were validated microscopically under 100x and the values were expressed as percent inhibition of entry of parasites. The 50% inhibitory concentration (IC₅₀) was determined.

2.6. Scavenging reaction between 1,1-diphenyl-2-picrylhydrazyl (DPPH) and antioxidant

The plant extract's ability to scavenge DPPH radical was determined according to the method described by Blois[19]. A total of

1.0 mg/mL of sample stock solutions of plant extract was prepared. This was further diluted to final concentrations of 250, 125, 62.5, 31.25, 15.62 and 7.81 µg/mL in methanol. A total of 1 mL of a 0.3 mM DPPH methanol solution was added to 2.5 mL solution of the extract of different concentrations and incubated at room temperature for 30 min in dark. The absorbance (Abs) of the mixture was measured at 520 nm and the percentage antioxidant activity (AA%) was calculated using the formula:

$$AA\% = 100 - [(Abs_{\text{sample}} - Abs_{\text{blank}}) \times 100] / Abs_{\text{control}}$$

3. Results

3.1. Preliminary phytochemical screening

The preliminary phytochemical screening revealed the presence of potent phytochemicals in the crude plant extracts. The investigated plants showed the presence of the following phytochemicals in the order of flavonoids > tannins, phenols > steroids and triterpenoids > glycosides > saponins. Flavonoids were detected in all the extracts. *Hyptis suaveolens* (*H. suaveolens*) (IC₅₀: 3.906 µg/mL) and *M. indica* (IC₅₀: 4.150 µg/mL) showed the presence of triterpenoids (Table 1).

3.2. HPTLC results

HPTLC separation of the crude extracts showed a number of phytochemicals, with *M. indica* having the highest number of compounds and *A. bilimbi* showing the least number of compounds (Figure 1). The chromatograms of all the extracts were scanned at 366 nm. The crude extracts of *M. indica*, *A. marmelos* (fruit), *F. religiosa*, *A. indica*, *M. oleifera*, *H. suaveolens*, *F. racemosa*, *A. marmelos* (leaf) and *A. bilimbi* revealed the presence of 9, 4, 2, 9, 5, 4, 2, 4 and 1 substance/s respectively. The results of the scan were shown in Figure 2.

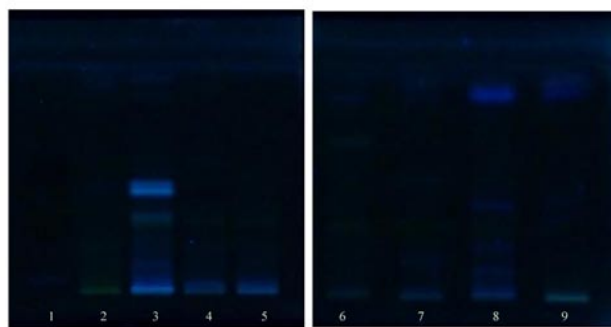


Figure 1. Chromatogram under UV- 366 nm.

Lane 1: *M. indica*, Lane 2: *A. marmelos* fruit, Lane 3: *F. religiosa*, Lane 4: *A. indica*, Lane 5: *M. oleifera*, Lane 6: *H. suaveolens*, Lane 7: *F. racemosa*, Lane 8: *A. marmelos* leaf and Lane 9: *A. bilimbi*.

3.3. In-vitro percentage inhibition of entry of parasites

After checking the antiplasmodial activity of the plant extracts, IC₅₀ value was calculated. The least IC₅₀ was exhibited by *M. oleifera* (IC₅₀: 3.906 µg/mL), *A. indica* (IC₅₀: 3.906 µg/mL), *H. suaveolens* (IC₅₀: 3.906 µg/mL), followed by *M. indica* (IC₅₀: 4.150 µg/mL) and *A. bilimbi* (IC₅₀: 4.881 µg/mL). Based on WHO recommendation, plants possessing IC₅₀ less than 5 µg/mL are considered as excellent. Artemisinin, currently used as antimalarial drug to treat patients suffering from malaria, was used as a standard, which displayed an IC₅₀ value of 3.906 µg/mL. The IC₅₀ in µg/mL of all the plant extracts screened for their antiplasmodial activity were as follows: *M. indica* (4.150), *A. marmelos* fruit (41.032), *F. religiosa* (5.265), *A. indica* (3.906), *M. oleifera* (3.906), *H. suaveolens* (3.906), *F. racemosa* (79.118), *A. marmelos* leaf (16.651), *A. bilimbi* (4.881).

3.4. Scavenging reaction between DPPH and antioxidant

Again *M. indica* and *H. suaveolens* exhibited the maximum antioxidant activity; even at the lowest concentration of 7.81 µg. Figure 3 shows the graph depicting the percentage antioxidant activity of all the crude extracts at varying concentrations. The effective concentration of plant extract required to scavenge

Table 1

Preliminary qualitative analysis.

Plant extracts	Compounds						
	Phenols	Tannins	Flavonoids	Glycosides	Saponins	Steroids and triterpenoids	Alkaloids
<i>M. indica</i>	+	+	+	+	+	Triterpenoid +nt	-
<i>A. marmelos</i> fruit	+	-	+	-	-	Steroid +nt	-
<i>F. religiosa</i>	+	+	+	-	-	Triterpenoid +nt	-
<i>A. indica</i>	+	+	+	+	-	-	-
<i>M. oleifera</i>	+	+	+	+	-	-	-
<i>H. suaveolens</i>	+	+	+	-	+	Triterpenoid +nt	-
<i>F. racemosa</i>	+	+	+	-	-	-	-
<i>A. marmelos</i> leaf	-	+	+	+	-	Triterpenoid +nt	-
<i>A. bilimbi</i>	-	-	+	+	-	Steroid +nt	-

“+” indicates presence and “-” indicates absence.

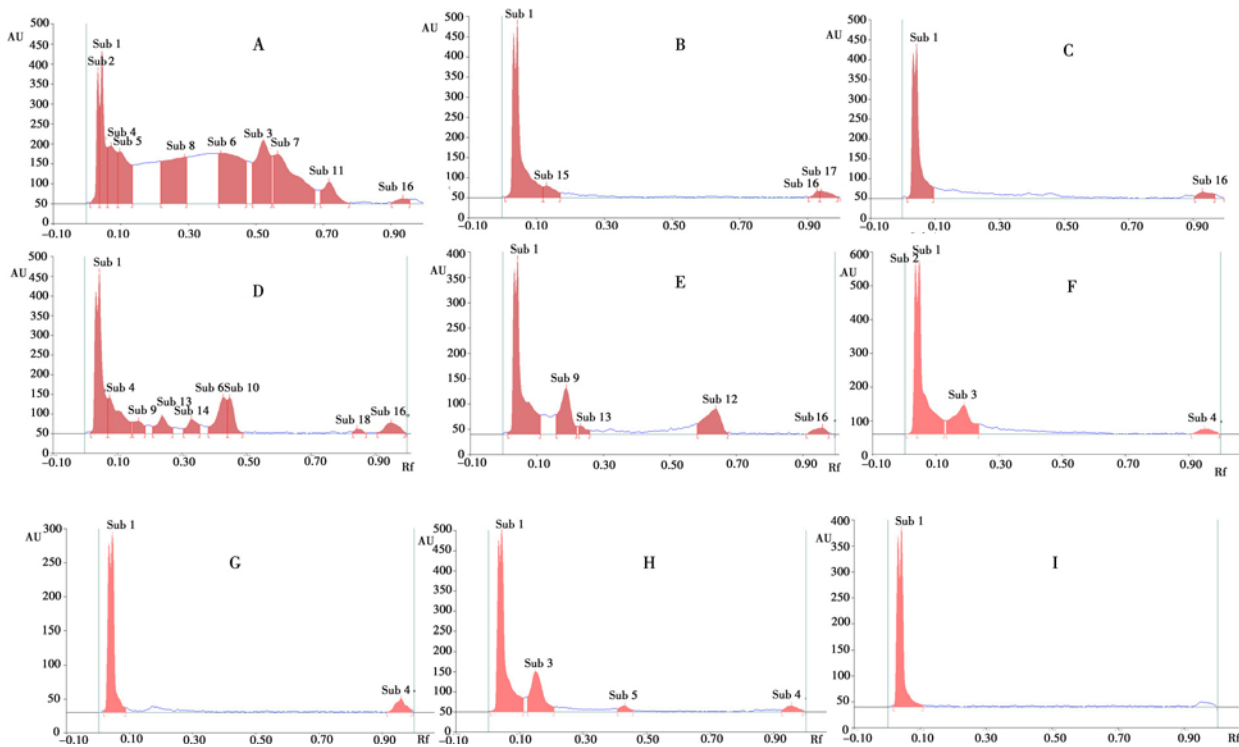


Figure 2. Chromatograms scanned at 366 nm with tungsten lamp using Camag Scanner [11] in combination with winCATS [12] software (Version 1.4.6.8121). A: Extract of *M. indica*, B: Extract of *A. marmelos* fruit, C: Extract of *F. religiosa*, D: Extract of *A. indica*, E: Extract of *M. oleifera*, F: Extract of *H. suaveolens*, G: Extract of *F. racemosa*, H: Extract of *A. marmelos* leaf, I: Extract of *A. bilimbi*. Sub: Substance.

the DPPH radical by 50% was calculated. The IC_{50} values of antioxidant activity of all the plant extracts expressed were as follows: *M. indica* (2.293 $\mu\text{g/mL}$), *A. marmelos* fruit (2.680 $\mu\text{g/mL}$), *F. religiosa* (2.733 $\mu\text{g/mL}$), *A. indica* (2.799 $\mu\text{g/mL}$), *M. oleifera* (2.871 $\mu\text{g/mL}$), *H. suaveolens* (2.683 $\mu\text{g/mL}$), *F. racemosa* (3.324 $\mu\text{g/mL}$), *A. marmelos* leaf (3.134 $\mu\text{g/mL}$), *A. bilimbi* (3.327 $\mu\text{g/mL}$).

4. Discussion

In the last decade there has been resurgence in search for new lead compounds from plants to treat malaria. However, only a few, out of 250 000 plant species, have been thoroughly investigated for their therapeutic abilities[20]. Plant based drugs by far have proved to be very effective remedies for the prevention as well as treatment of various diseases and are known to provide the lead compound or the backbone for the various drugs currently in use[21]. The present study is aimed at ethno-directed sampling approach for screening and identification of potential plant species for antiplasmodial activity.

Among the nine plant species selected on the basis of their ethnobotanical uses, *M. indica* and *H. suaveolens* were found to be highly potential sources for isolation and/ or development of lead compounds with antiplasmodial activity. The IC_{50} value of artemisinin, which was used as a standard, was also found similar to that of *M. indica* and *H. suaveolens*. Interestingly, *M. indica* has been already reported for its antiplasmodial activity[22] as well as antipyretic effect, which was proved by Awe *et al*[23], by demonstrating an *in-vivo* test of stem bark extract against *Plasmodium yoelii nigeriensis* in mice[4], while it is reported that a compound named 13 alpha-epi-dioxiabiet-8(14)-en-18-ol, isolated from petroleum ether leaf extract of *H. suaveolens* showed very high antiplasmodial activity, having an IC_{50} value of 0.1 $\mu\text{g/mL}$ [24]. In another study, *H. suaveolens* ethanolic leaf extract also

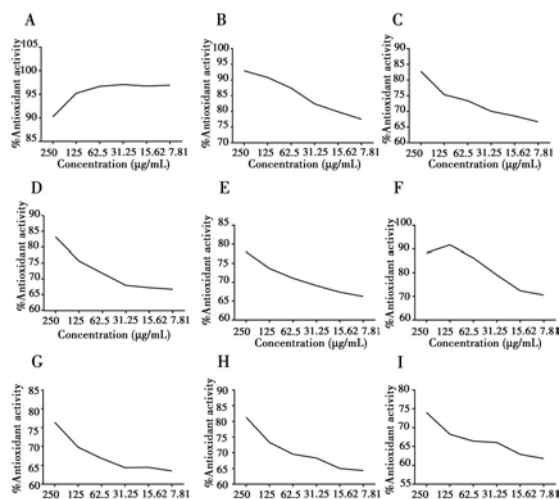


Figure 3. Percentage antioxidant activity of all crude extracts at varying concentrations.

(A) *M. indica*; (B) *A. marmelos*; (C) *F. religiosa*; (D) *A. indica*; (E) *M. oleifera*; (F) *H. suaveolens*; (G) *F. racemosa*; (H) *A. marmelos*; (I) *A. bilimbi*.

displayed good antiplasmodial activity against *Plasmodium berghei*, with 51.05% suppression. A compound named dehydroabietinol, isolated from *H. suaveolens*, was reported to display an IC₅₀ value ranging between 26–27 µM, against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*[25].

In the present study using the crude whole plant extract, the IC₅₀ value obtained for *H. Suaveolens* was 3.906 µg/mL. Identification and purification of active principle from the crude extract may lead to either reduction or increase in the IC₅₀ value. In another study by Bagavan *et al.*, hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Citrus sinensis* (peel), *Leucas aspera*, *Ocimum sanctum*, *Phyllanthus acidus* (leaf), *Terminalia chebula* (seed) were tested for their antimalarial activity against 3D7 strain of *P. falciparum*. The IC₅₀ values ranged from 4.76 µg/mL to above 88.03 µg/mL[26]. There are various reports showing a large number of plants screened for their antiplasmodial activity against *P. falciparum*[26–30]. Dr. Duke's Database has reported antimalarial as well as antipyretic activity of *M. indica*, *A. marmelos*, *H. suaveolens* and *A. bilimbi*. *A. indica* is reported to possess antipyretic effect, while *M. oleifera* and *A. bilimbi* are known to possess antimalarial property. *M. oleifera* is reported to possess antipyretic effect[31]. Antiplasmodial activity of the aqueous extract and ethanol extract (twig and whole plant), of *M. oleifera* was checked using 3D7 strain of *P. falciparum*. Aqueous extract demonstrated IC₅₀ value of 43.65 µg/mL, while ethanol extract showed an IC₅₀ value of 15.13 µg/mL[32]. In yet another study, the crude ethanolic and *n*-hexane extracts of *M. oleifera* leaves showed a promising *in-vitro* antiplasmodial activity ranging from 74.7% to 95.6% inhibition of parasitaemia[33].

Artemisia annua L. (Asteraceae) is a native to China. It was used traditionally to treat fever. Later on it was identified as an antimalarial. In 1972, artemisinin, the active component was isolated and identified by the Chinese. Artemisinin chemically is a sesquiterpene peroxide, an endoperoxide compound. From this lead compound, various derivatives were then produced[34,35]. The plants which have manifested the best antiplasmodial activity (*M. indica* and *H. suaveolens*) have triterpenoids as the major phytochemical component. It is therefore possible that the antiplasmodial activity exhibited by these plants is also probably due to the presence of these phytoconstituents.

The effectiveness of a substance in inhibiting a specific biochemical or biological function is called its IC₅₀ value. It indicates the amount of a particular drug required to inhibit a biological or biochemical process by half. Lower the IC₅₀ value, lesser the side effects of the drug. In the present study, plant extracts of *M. indica* and *H. suaveolens* showed low IC₅₀ values. Based on the literature survey and the results obtained in the present study, ethno-directed plant selection approach seems to have higher efficacy in identifying potential plant species with therapeutic properties, rather than the random selection approach. Plants possess a wide range of metabolites such as alkaloids,

glycosides, phenols, terpenoids, flavonoids and various other such phytochemicals. These metabolites are known to be a rich source of antioxidants[36]. In patients suffering from *P. falciparum* infection, there is an increase in the level of lipid peroxidation. This happens due to the release of reactive oxygen species from the infected erythrocytes. The main reason for anaemic condition in patients suffering from malaria is due to this oxidative stress. Higher the parasitaemia, higher the amount of reactive oxygen species released[37]. The present study showed that *M. indica* and *H. suaveolens* plant extracts possess high antioxidant activity. On the other hand, *A. bilimbi* showed good antiplasmodial activity, but weak antioxidant activity. A drug with antimalarial as well as antioxidant property can help in checking malarial anaemia effectively[37]. Further studies on isolation and characterization of active compounds from the selected plant species may prove to be useful.

The antioxidant activity of the crude extract, we can assume, can counteract the oxidative damage caused due to the malaria parasite. Moreover the IC₅₀ value exhibited by the crude extract of *M. oleifera*, *A. indica* and *H. suaveolens*, when compared with artemisinin are found to be promising. Further investigations to explore and identify the active principle/s by bioassay-guided fractionation from *M. oleifera*, *A. indica*, *M. indica*, *H. suaveolens* and *A. bilimbi*, are in progress.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The authors are grateful to P. G. Department of Biosciences, Centre of Advanced Studies, Sardar Patel University and Department of Zoology, Biomedical Technology and Human Genetics, School of Sciences, Gujarat University, for providing the necessary facilities and requirements throughout the research.

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