

Research Article

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Anti-plasmodial potential of selected medicinal plants and a compound Atropine isolated from *Eucalyptus obliqua*

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Abstract: The present research study was aimed to investigate the efficiency of selected medicinal plants against *Plasmodium vivax*. Crude methanolic extracts from the seeds of leaves of *Datura stramonium*, *Parthenium hysterophorus*, *Calotropis procera*, and *Dodonaea viscosa* were prepared. In addition, Atropine was also isolated from alkaloid components of *Eucalyptus obliqua* to evaluate their *in vitro* anti-plasmodial effects. It was observed that proguanil (positive control) and Atropine displayed strong anti-plasmodial activity (94.04 and 68.02%, respectively) against *P. vivax* at 0.1 mg/mL concentration while the leaf extracts of other medicinal plants did not exhibit any notable anti-plasmodial activity. It was concluded that alkaloids of *E. obliqua* plant's extracts were rich in anti-plasmodial compound Atropine, which exhibit a remarkable anti-plasmodial activity against *P. vivax*. Anti-plasmodial action of medicinal plants are attributed to these phytochemicals. *In vitro* studies using medicinal plant's extracts and standardized methods will help to make more powerful and cost-effective anti-plasmodial compounds.

Keywords: medicinal plants, methanolic extract, anti-plasmodial activity, Atropine, proguanil

1 Introduction

Medicinal plants are being used as a treatment for several microbial infections for hundreds of years, but they are also being used as health and growth promoters in animal feed [1]. Many medicinal plants are extensively used in the preparation of therapeutic drugs to treat a variety of microbial infections [2]. Due to improper diagnosis and an elevated exposure of antibiotics and antimalarial compounds, the risk of drug resistance is alarmingly increased [3,4].

Malaria is an erythrocytic blood infection caused by a hemo-parasite of genus *Plasmodium* [5]. About one-half of the world's population is at risk of malaria infection [6]. Following the Sustainable Development Goals 2030, World Health Organization has specified the Global Technical Strategy for the eradication of malaria worldwide. This strategy is intended to reduce the death rate caused by malaria infection up to 90%. It is also intended to get 35 countries free of malaria infection and also to prevent malaria comeback in 20 countries that were malaria-free in 2001 [7]. Building solid alliances and government commitment, developing evidence-based policy for malaria eradication in various transmission zones, and investing in effective control measures and surveillance systems are all part of this strategy [8].

Multi-drug resistance in pathogens is increasing nowadays, which leads to difficulty in treating life-threatening viral, bacterial, and parasitic infections [9]. Alteration in drug binding sites and enzymes involved in drug inactivation is responsible for evolving resistance against commercial drugs. Alterations of enzymatic pathways are also considered to contribute to the drug resistance in microorganisms [10].

For thousands of years, medicinal plants are being used to cure a variety of diseases in humans and animals. Medicinal plants are traditionally being used for various disorders in Arabic herbal studies, such as bacterial infections, conjunctivitis, asthma, GIT dysfunctions, and hypertension. The therapeutic impact of medicinal plants is recently a hot topic. Research is being conducted on

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medicinal plants to cash its pharmacological properties to treat cancer and gastrointestinal diseases. Medicinal plants are also an active area of research in the recent era [11]. It is also reported that the oil of *Eucalyptus obliqua* decreases the level of free radicals and has a high impact on chemokines such as prostaglandins and cytokines [12].

Atropine is a naturally occurring compound found in most of the medicinal plants. It is listed in the essential medicines list by the WHO due to its dire importance and medical uses. The most common medical effect of Atropine is in gastric, bronchial, and salivary secretions [13].

The leaves and roots of *Datura stramonium*, *Calotropis procera*, and *Dodonaea viscosa* also traditionally used as folk medicines for the treatment of different diseases including skin infections, healing of wounds, a bronchodilator, rheumatic pains, skin ulcers, skin burns, painful menstruation, antispasmodic, and anti-diabetic [14–16]. There is a massive decrease in options for treating bacterial and parasitic infections worldwide; therefore, there is a dire need to search for new plant-based remedies to cope with such infections effectively. Therefore, keeping in view the importance of medicinal plants, the present study was designed to investigate the efficiency of selected medicinal plants against *Plasmodium vivax* and *Staphylococcus aureus*.

2 Materials and methods

2.1 Collection of medicinal plants

Fresh leaves of *E. obliqua*, *D. stramonium*, *P. hysterothorus*, *C. procera*, *P. hysterothorus*, and *D. viscosa* were collected

from the hills of Kohat Developing Authority (KDA), Kohat (coordinates latitude: 33.60 and longitude: 71.46) (Figure 1). Medicinal plants were identified by the experts of the Department of Botany, Kohat University of Science and Technology, Kohat KP, Pakistan, vide voucher numbers C-0138, D-3443, D-987, and P-334 for *C. procera*, *D. stramonium*, *D. viscosa*, and *P. hysterothorus*, respectively.

Atropine was isolated and purified from alkaloids of *E. obliqua*. Plant samples were transported to the Molecular Parasitology Laboratory, Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa, Pakistan.

2.2 Preparation of methanolic extract

Crude methanolic extract was prepared in 100% methanol from leaves of *E. obliqua*, *D. stramonium*, *P. hysterothorus*, *C. procera*, *P. hysterothorus*, and *D. viscosa* using standard protocol as described by Nanasombat and Lohasupthawee [17]. After preparation of extracts, five different concentrations, i.e., 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL were prepared using distilled water as a solvent.

2.3 Isolation and purification of Atropine through Gradient Solvent Systems and high performance liquid chromatography (HPLC)

Gradient Solvent Systems were designed to isolate the antiplasmodial components in alkaloids of *E. obliqua* crude

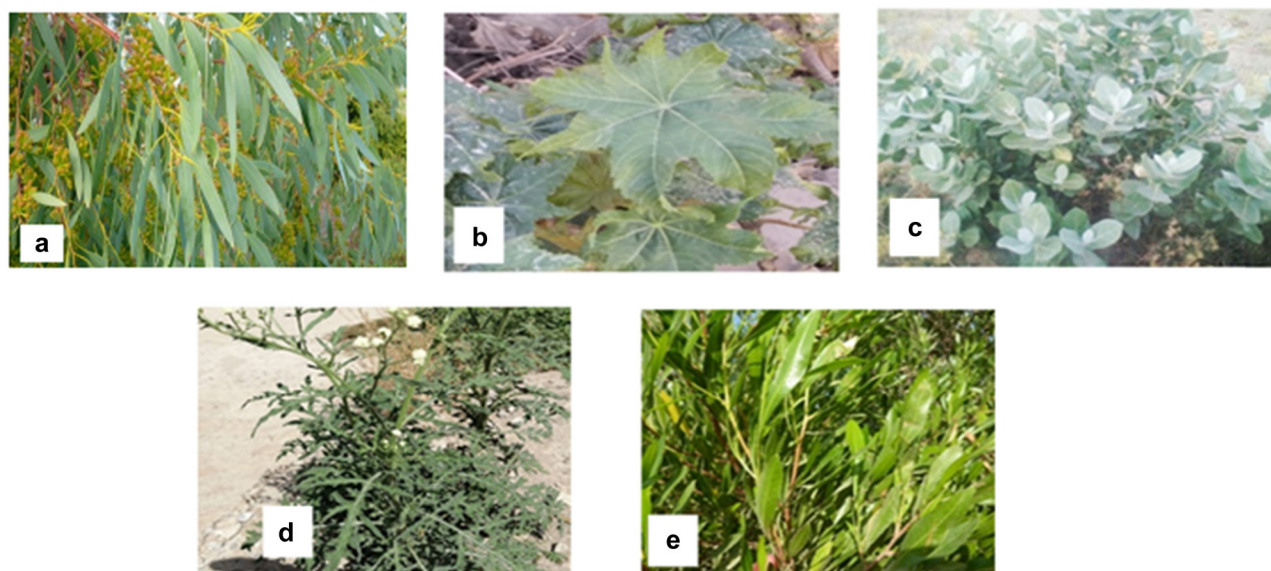


Figure 1: Medicinal plants used in the preparation of crude methanolic extract: (a) *E. obliqua*, (b) *D. stramonium*, (c) *C. procera*, (d) *P. hysterothorus*, and (e) *D. viscosa*.

extract through Silica Gel Column Chromatography. Various concentrations of *n*-hexane and methanol were prepared, and extracts were yielded by the repeated experimentation. Finally, samples were subjected to HPLC and Atropine was isolated (Figure 2).

2.4 Plasmodial culture collection

Prepared culture of *P. vivax* was obtained from Molecular Parasitology Laboratory, KUST, Kohat, Khyber Pakhtunkhwa, Pakistan, while anti-plasmodial activity of crude methanolic extract of selected medicinal plants was evaluated using standard protocols as described by Nyandwaro et al. [18].

2.5 In vitro anti-plasmodial activity

A total of 32 wells were prepared in microtiter plates containing agar medium. Each well was filled with parasitized RBCs. Furthermore, five concentrations of Proguanil were used as a positive control based on field survey. It was revealed in field survey that proguanil products were in trending use and giving good results against malaria infection in the first five wells, while five concentrations of plant extracts, i.e., Atropine, *D. stramonium*, *P. hystero-phorus*, *P. hystero-phorus*, and *D. viscosa* were used as experimental group. In addition, distilled water was used as negative control. After the incubation period of 48 h, the supernatants from each well were removed, and smear were prepared for further anti-plasmodial assay

as per protocol of WHO [19]. Maturation and inhibition percentages were evaluated using the following formula as described by Kwansa-Bentum et al. [20]. However, mean inhibition, standard error, and standard deviation were calculated using Statistix 9 software:

$$\text{Maturation\%} = \frac{\text{No. of developed schizonts for Experimental group}}{\text{No. of developed schizonts for control}} \times 100 \quad (1)$$

$$\text{Inhibition\%} = 100 - \text{Maturation\%}. \quad (2)$$

3 Results

In the current research study, it was observed that methanolic extracts of proguanil and Atropine exhibited 94.04 and 68% inhibition, respectively, against *P. vivax* with the LD₅₀ 0.068. On the other hand, leaf extracts of *D. stramonium*, *C. procera*, *P. hystero-phorus*, and *D. viscosa* did not exhibit any notable anti-plasmodial activity, i.e., 8.04, 4.02, 4.59 and 2.12% inhibition, respectively, against *P. vivax* as shown in Table 1.

4 Discussion

The study was aimed to investigate the *in vitro* anti-plasmodial potential of Atropine, *D. stramonium*, *C. procera*, *P. hystero-phorus*, and *D. viscosa* extracts against *P. vivax*. The results revealed that the methanolic extracts of all these medicinal plants *except* Atropine exhibited a minimal anti-plasmodial activity against *P. vivax*.

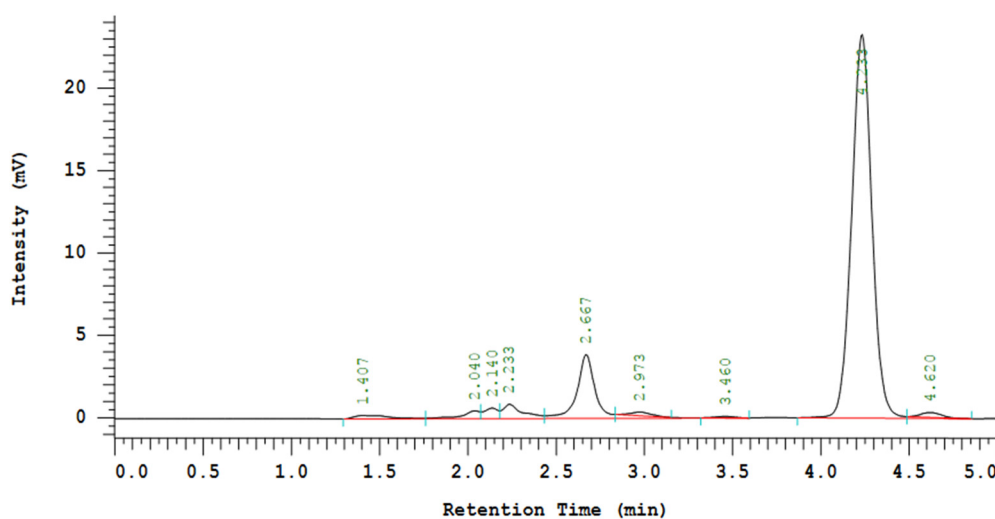


Figure 2: HPLC peak of isolate compound “Atropine.”

Atropine is an amalgamation of L-hyoscyamine and D-hyoscyamine which is identified as a tropane alkaloid. L-Hyoscyamine is responsible for most of its physiological effects. Pharmacologically it is very important as it binds to muscarinic acetylcholine receptors and induce pharmacological actions as antimuscarinic mediator. Stereoselective metabolism of Atropine is also reported in various studies. Sulfate (monohydrate) is the most common Atropine which is used in the medicines. About 60% of the Atropine, when taken, are excreted from the body unchanged in the urine, rest of the 40% become hydrolyzed. Glands regulated by the parasympathetic nervous system perform their activity in “rest and digest rule.” Generally Atropine counters the rest and digest activity [21].

As reported by our previous studies [22] the alkaloid components of *E. obliqua* exhibit a remarkable anti-plasmodial potential. It was concluded that alkaloids contain some anti-plasmodial compounds which are responsible

for the mortalities of the *P. vivax* *in vitro*. In addition, several studies have already been conducted to evaluate the anti-microbial activity of medicinal plants [23,24].

Moreover, *in vitro* studies using comparable extracts and standardized methods are required to make more definitive claims regarding medicinal plants and its derivatives’ powerful anti-plasmodial effects in pharmacological formulations.

Medicinal plant-based research is seen as a universal solution to inhibit the pathogenesis of microorganisms. Genetic variations and polymorphism in genes of the *Plasmodium* lead to the drug resistance in *Plasmodium* species [25]. Leaf extracts of *D. stramonium* and *C. procera* exhibited a noteworthy anti-plasmodial activity, i.e., 8.04, and 4.02% inhibition, respectively, against *P. vivax*. The current findings on a crude extract of leaves also support prior results, demonstrating the widespread usage of medicinal plants as an anti-malarial agent by Western Cameroon’s

Table 1: Inhibition percentage of proguanil, Atropine, *D. stramonium*, *C. procera*, *P. hysterophorus*, and *D. viscosa* crude extracts against *P. vivax*

Drugs/samples	Concentration (mg/mL)	Schizonts in experimental group	Schizonts in control group	Maturation (%)	Inhibition (%)
Proguanil	0.02	85.78 ± 1.9	251.6	34.08	65.92
	0.04	75.89 ± 2.9		30.15	69.85
	0.06	51.44 ± 2.9		20.44	79.56
	0.08	30.11 ± 2.5		11.96	88.04
	0.1	15.00 ± 2.5		5.96	94.04
Atropine	0.02	114.56 ± 1.5	151.3	75.70	24.30
	0.04	113.67 ± 0.4		75.11	24.89
	0.06	104.78 ± 1.5		69.24	30.76
	0.08	78.00 ± 1.2		51.54	48.46
	0.1	47.67 ± 0.3		31.50	68.50
<i>D. stramonium</i>	0.02	196.78 ± 2.5	251.6	95.19	4.81
	0.04	185.33 ± 2.5		93.64	6.36
	0.06	172.89 ± 1.3		93.7	6.3
	0.08	112.89 ± 2.9		92.86	7.14
	0.1	65.33 ± 2.0		91.96	8.04
<i>C. procera</i>	0.02	248.56 ± 2.0	251.6	98.76	1.24
	0.04	248.00 ± 1.7		98.54	1.46
	0.06	246.22 ± 1.3		97.84	2.16
	0.08	242.11 ± 0.8		96.20	3.80
	0.1	241.56 ± 1.4		95.98	4.02
<i>P. hysterophorus</i>	0.02	248.00 ± 0.9	251.6	98.54	1.46
	0.04	243.89 ± 1.2		96.91	3.09
	0.06	243.11 ± 0.5		96.60	3.40
	0.08	240.11 ± 1.3		95.41	4.59
	0.1	241.56 ± 1.5		95.98	4.02
<i>D. viscosa</i>	0.02	247.11 ± 1.1	251.6	98.19	1.81
	0.04	248.89 ± 0.7		98.90	1.10
	0.06	246.89 ± 0.99		98.10	1.90
	0.08	249.00 ± 1.2		98.94	1.06
	0.1	246.33 ± 1.4		97.88	2.12

endogenous traditional healers [26]. Xu et al. [27] reported alkaloids, flavonoids, saponins, and tannins during preliminary phytochemical screening of leaf extracts of medicinal plants and concluded that the extract's anti-plasmodial action might be attributed to one or more groups of these components.

In the current study, *in vitro* anti-plasmodial activity of medicinal plant's extracts is also in agreement with the study of Satish et al. [26], which revealed that *D. stramonium* had anti-plasmodial potential against drug-resistant *Plasmodium* species. The anti-plasmodial activity of *E. obliqua* was due to the presence of alkaloids which contain Atropine, i.e., an anti-plasmodial compound. Further, *in vivo* studies are required to understand the detailed mechanism of anti-plasmodial activity of active anti-plasmodial compounds in other selected medicinal plant extracts. In this study, the anti-plasmodial efficacy of *P. hystrophorus* was not found, which is in quite contradiction to the study of Ahmed et al. [28] who reported the *in vitro* anti-plasmodial potential of *P. hystrophorus* on chloroquine-sensitive strains of *Plasmodium falciparum*. The possible reason might be that Ahmed et al. [28] used whole plant in the preparation of extracts to perform the *in vitro* activity, while in the current study, only leaves of *P. hystrophorus* and *D. viscosa* were used in the preparation of extracts. Similarly, no satisfactory antimalarial activity of *C. procera* was observed in the current study, which is in contradiction to the study of Adejoh et al. [29], who used phosphate buffer extract from the latex of *C. procera* and evaluated the *in vivo* antimalarial activity on mice. Studies suggested that medicinal plants from *Eucalyptus* species are traditionally being used for the treatment of malaria infection in various parts of Africa [30]. One of the species, i.e., *Eucalyptus globulus* has also traditional popularity in healing malaria infection in Brazil [31]. Moreover, *C. procera*, *D. stramonium*, *D. viscosa*, and *P. hystrophorus* are being used traditionally to repel the malaria vector [32,33].

5 Conclusions

In the current study it was concluded that Atropine isolated from *E. obliqua* extract displayed anti-plasmodial activity against *P. vivax* at all concentrations; however, Atropine is reported for the first time for its anti-plasmodial potential particularly for *P. vivax*. A strong anti-plasmodial activity was found at higher concentration, i.e., 0.1 mg/mL. On the other hand, it was also concluded that the leaf extracts of *D. stramonium*, *C. procera*, *P. hystrophorus*, and *D. viscosa* did not reveal any prominent

anti-plasmodial activity against *P. vivax*. Moreover, it was suggested that further *in vitro* and *in vivo* studies are needed to explore the medicinal plants and its derivatives' powerful anti-plasmodial and anti-bacterial effects in future. Furthermore, the mode of action of Atropine and the detailed study of the target site of this compound is needed to be investigated.

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Conflict of interest: All authors declare no conflict of interest regarding this manuscript as well as research study.

Ethical statement: Ethical consideration for this study was taken from Research Ethical Committee of KUST, Kohat, Khyber Pakhtunkhwa, Pakistan.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- [1] Oluwafemi RA, Olawale I, Alagbe JO. Recent trends in the utilization of medicinal plants as growth promoters in poultry nutrition – a review. *Res Agric Vet Sci.* 2020;4(1):5–11.
- [2] Akram M, Riaz M, Noreen S, Shariaty MA, Shaheen G, Akhter N, et al. Therapeutic potential of medicinal plants for the management of scabies. *Derm Ther.* 2020 Jan;33(1):e13186.
- [3] Bloland PB, World Health Organization. Drug resistance in malaria. World Health Organization; 2001.
- [4] Knöppel A, Näsvall J, Andersson DI. Evolution of antibiotic resistance without antibiotic exposure. *Antimicrob Agents Chemother.* 2017 Nov 1;61(11):e01495–17.

- [5] Breman JG, Alilio MS, Mills A. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. *Am J Trop Med Hyg.* 2004 Aug 1;71(2 Supp):1–5.
- [6] World Health Organization. The world health report 2006: working together for health. World Health Organization; 2006 Mar 23.
- [7] World Health Organization. Global technical strategy for malaria 2016–2030. World Health Organization; 2015 Nov 4.
- [8] Global health program, A report of Bill and Melinda Gates Foundation. Malaria strategy over-view; 2015.
- [9] El Moussaoui A, Bourhia M, Jawhari FZ, Salamatullah AM, Ullah R, Bari A, et al. Chemical profiling, antioxidant, and antimicrobial activity against drug-resistant microbes of essential oil from *Withania frutescens* L. *Appl Sci.* 2021;11(11):5168.
- [10] Xia J, Gao J, Tang W. Nosocomial infection and its molecular mechanisms of antibiotic resistance. *Biosci Trends.* 2016;10(1):14–21.
- [11] Sarwar A, Latif Z. GC–MS characterisation and antibacterial activity evaluation of *Nigella sativa* oil against diverse strains of *Salmonella*. *Nat Prod Res.* 2015 Mar 4;29(5):447–51.
- [12] Gholamnezhad Z, Keyhanmanesh R, Boskabady MH. Anti-inflammatory, antioxidant, and immunomodulatory aspects of *Nigella sativa* for its preventive and bronchodilatory effects on obstructive respiratory diseases: a review of basic and clinical evidence. *J Funct Foods.* 2015 Aug 1;17:910–27.
- [13] Lide DR, editor. Handbook of data on organic compounds. 2. Compounds 5001–10000, Ben-Cha. Boca Raton, Florida: CRC Press; 1994.
- [14] Parrotta JA. Healing plants of peninsular India. Wallingford, United Kingdom: Cabi Publishing; 2001.
- [15] Khan MF, Alqahtani AS, Almarfadi OM, Ullah R, Nasr FA, Noman OM, et al. The reproductive toxicity associated with *Dodonaea viscosa*, a folk medicinal plant in Saudi Arabia. *Evid-Based Complement Altern Med.* 2021;2021:1–9.
- [16] Alqahtani AS, Ullah R, Shahat AA. Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia. *Evid-Based Complement Altern Med.* 2022;2022:7123521.
- [17] Nanasombat S, Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. *Curr Appl Sci Technol.* 2005;5(3):527–38.
- [18] Nyandwaro K, Oyweri J, Kimani F, Mbugua A. Evaluating antiplasmodial and antimalarial activities of soybean (*Glycine max*) seed extracts on *P. falciparum* parasite cultures and *P. berghei*-infected mice. *J Pathog.* 2020 Feb 17;2020:1–8.
- [19] World Health Organization. Malaria microscopy quality assurance manual-version 2. World Health Organization; 2016 Apr 5.
- [20] Kwansa-Bentum B, Agyeman K, Larbi-Akor J, Anyigba C, Appiah-Openg R. *In vitro* assessment of antiplasmodial activity and cytotoxicity of *Polyalthia longifolia* leaf extracts on *Plasmodium falciparum* strain NF54. *Malar Res Treat.* 2019;2019:6976298.
- [21] Van der Meer MJ, Hundt HK, Müller FO. The metabolism or atropine in man. *J Pharm Pharmacol.* 1986 Oct;38(10):781–4.
- [22] Zareen S, Khan SN, Adnan M, Haleem S, Ali R, Alnomasy SF. Antiplasmodial potential of *Eucalyptus obliqua* leaf methanolic extract against *Plasmodium vivax*: an *in vitro* study. *Open Chem.* 2021 Jan 1;19(1):1023–8.
- [23] Sarwar A, Latif Z. GC–MS characterisation and antibacterial activity evaluation of *Nigella sativa* oil against diverse strains of *Salmonella*. *Nat Prod Res.* 2015 Mar 4;29(5):447–51.
- [24] Senthilnathan K, Ilango P, Abirami T, Vummidi VA, Mahalingam AP, Reddy VK. Evaluation of antibacterial activity of *Nigella sativa* seed extract against *Porphyromonas gingivalis* and *Prevotella intermedia*. *J Interdiscip Dent.* 2020 May 1;10(2):51.
- [25] Khan SN, Ali R, Khan S, Rooman M, Norin S, Zareen S, et al. Genetic diversity of polymorphic marker merozoite surface protein 1 (*Msp-1*) and 2 (*Msp-2*) genes of *Plasmodium falciparum* isolates from malaria endemic region of Pakistan. *Front Genet.* 2021;12:751552.
- [26] Satish PVV, Santha Kumari D, Sunita K. Antimalarial efficacy of *Datura stramonium* against chloroquine sensitive *Plasmodium falciparum* 3d7 strain. *Eur J Pharm Med Res.* 2018;5(3):287–94.
- [27] Xu R, Snyder JK, Nakanishi K. Robustadiols A and B from *Eucalyptus robusta*. *J Am Chem Soc.* 1984 Feb;106(3):734–6.
- [28] Ahmed EH, Nour BY, Mohammed YG, Khalid HS. Antiplasmodial activity of some medicinal plants used in Sudanese folk-medicine. *Environ Health Insights.* 2010 Jan;4:EH1-S4108–6.
- [29] Adejoh J, Inyang BA, Egua MO, Nwachukwu KC, Alli LA, Okoh MP. In-vivo anti-plasmodial activity of phosphate buffer extract of *Calotropis procera* latex in mice infected with *Plasmodium berghei*. *J Ethnopharmacol.* 2021 Sep 15;277:114237.
- [30] Connelly MP, Fabiano E, Patel IH, Kinyanjui SM, Mberu EK, Watkins WM. Antimalarial activity in crude extracts of Malawian medicinal plants. *Ann Trop Med Parasitol.* 1996 Jan 1;90(6):597–602.
- [31] Milliken W. Traditional anti-malarial medicine in Roraima, Brazil. *Econ Bot.* 1997 Jul;51(3):212–37.
- [32] Bansal SK, Singh KV, Sharma S, Sherwani MR. Laboratory observations on the larvicidal efficacy of three plant species against mosquito vectors of malaria, dengue/dengue hemorrhagic fever (DF/DHF) and lymphatic filariasis in the semi-arid desert. *J Environ Biol.* 2012 May 1;33(3):617–21.
- [33] Afolabi OJ, Simon-Oke IA, Elufisan OO, Oniya MO. Adulticidal and repellent activities of some botanical oils against malaria mosquito: *Anopheles gambiae* (Diptera: Culicidae). *Beni-Suef Univ J Basic Appl Sci.* 2018 Mar 1;7(1):135–8.