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Original Research Article

Gas Chromatography-Mass Spectrometry Analysis and Antimalarial Activity of *Salix ledermannii* Ethanol Leaves Extracts

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ABSTRACT

Although malaria is curable, it remains the leading cause of mortality in Nigeria. Eliminating malaria is a pressing concern due to the challenge of drug-resistant *Plasmodium* parasites. Therefore, there is an urgent need for antimalarial agents that are affordable, safe, and effective against drug-resistant strains. The antimalarial potential of *Salix ledermannii* ethanol leaf extracts and their effect on PCV and haemoglobin levels, as well as the phytochemical compositions of the extract, were evaluated in this study using *Plasmodium berghei* infected mice as models. 0.2 ml Distilled water (Dw) was used as the negative control, while 5 mg/kg chloroquine and 1.2 mg/kg pyrimethamine served as the positive control. The phytochemical composition was analysed using standard chemical tests and GC-Ms techniques. Compared to the negative control, *S. ledermannii* showed significant chemo-suppression at 100 mg (44.38 %) and 300 mg/kg (46.75 %). Also, 5 mg/kg-chloroquine and treatment at 300 mg/kg significantly inhibited parasitaemia compared to the control group in the curative test model (p<0.05). The prophylactic test did not differ significantly (p>0.05) across treatment groups. The crude extract contained alkaloids, tannins, steroids, terpenes, anthraquinones, phenols, and saponins, and GC-Ms analysis revealed forty-three (43) known compounds, with benzoic acid (27.21%), phenol (15.22%), β-D-Glucopyranose (12.29%), Salicylalcohol (11.88%), 1-Butanol, 3-methyl (6.93%), Catechol (6.35%) and -ethyl-5,6-dihydo-2H-pyran-2-one (5.48%) having the highest concentrations. *S. ledermannii* poses no adverse effect on PCV and H.B. levels of treated mice and exhibits significant antimalarial properties. Thus, this plant can serve as a novel lead for compounds in the next-generation antimalarial drugs.

***Keywords*:** Antimalaria activities, *Salix ledermannii*, *Plasmodium berghei*, Gas chromatography-mass spectrometry.

Introduction

Malaria, caused by the protozoan parasite *Plasmodium*, is a deadly disease transmitted by the female *Anopheles* mosquito via an infective bite. The disease affects both humans and other vertebrate hosts.[1] Five known *Plasmodium* species that cause malaria infection in humans. These are *Plasmodium malariae*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium knowlesi*.[2] Infections also occur through the transfusion of infected blood (transfusion malaria), mother-to-child transmission (congenital malaria), and use of contaminated syringes, especially in drug addicts (Mainline malaria), which are known as trophozoite-induced malaria. The symptoms can include a range of debilitating effects such as headache, fever, chills, joint pain, vomiting, anaemia, yellowing of the skin and eyes (jaundice), dark urine, retinal damage, and, in severe cases, seizures and convulsions.[3]

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The classic symptom of one infected with malaria is paroxysm, a periodic episode characterised by a recurring cycle of chills and shivering. Depending on the parasite species one is infected with, the symptoms may present as tertian fever if the infecting parasite is *P. vivax* and *P. ovale*, with the episodes occurring every two days, or quartan fever if infected with *P. malariae* with episodes occurring every three days. Conversely, malaria infection may result in recurrent fevers with manifestation every 36–48 hours or a persistent low-grade common in infections with *P. falciparum*.[4] *P. falciparum* is the most critical plasmodium parasite known to cause the severe and deadly form of malaria, and most malaria-related illnesses and deaths are attributed to this parasite[5], with the symptoms of infection arising 9–30 days after infection.[6]

ledermannii is a shrub found around rivers, streams, and even drier environments. It grows to heights of up to 6-10 meters and possesses oblong leaves arranged in a spiral pattern that may appear silvery at times.[7,8] Documented evidence has shown that *S. ledermannii* has been used in traditional medicine to cure joint pains, inflammation, fevers, and other ailments. Chiefly, in the plant is the compound salicin, which is converted in the gut into salicyl alcohol and D-glucose, providing its therapeutic effects.[9] Plants generally pose as an invaluable natural resource to man. Apart from being used as food, their role as sources of natural cures for several illnesses has been documented since ancient times. Plants are the cornerstone of modern-day medicine as they have been used in pharmaceutical industries owing to the presence of diverse chemical elements (mostly secondary metabolites) in them that are responsible for their curative/protective properties.[10] However, the challenge comes with knowing the suitable methods to extract the correct compound. Several methods have been used to extract plant-based compounds. Still, the Gas chromatography-mass spectrometry

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analysis (GC-MS method) is considered a simple, rapid, and economical method used in extraction. It has the advantage of reduced solvent consumption and a wide application, such as extracting compounds from herbs used in cosmetics, drugs, pharmaceutical or food industry, and environmental and forensic studies.[11]

Recently, the concern over the continued resistance to chloroquine and other synthetic antimalarial medications by *P. falciparum* has become worrisome. Research is currently aimed at finding more effective and affordable antimalarial compounds12,13, which are particularly effective against parasites during the blood stage of infection. In view of this, investigating the potential of plant-based compounds as effective treatments for malaria has become pertinent. Therefore, we conducted this study to evaluate the effectiveness of *ethanol* leaf extract of *S. ledermannii* as an antimalarial agent that could be used in place of the existing drugs to which resistance has been confirmed.

Materials and Methods

Chemicals, reagents, and equipment

Analytical grade chemicals were used throughout this experiment: EDTA, Turk Solution (6%), Leishman stain (pH 6.8), Hematocrit, 8-bucket centrifuge (Model Number: 800-1, Holamedicals. Beaker, Test Tubes, Plastic cannula, ETDA bottles, Syringe-5 mls and 10 mls, Microscope (Light Microscope, Motec SFC-28 series, by Motec Company), Whatman filtration Paper (Whatman 1001-0901), Digital sensitive weighing balance- Telodo PB602 by Mettler Telodo, Transparent plastic cage 20cm x 18cm x 14cm. Microscope slides and cover glass by Four E's Scientific were utilised throughout this research. Beakers, Test tubes, Plastic cannulas, a Light, Whatman No. 1 filter paper, Telodo weighing balance, and plastic cages.

Collection and extraction of plant materials

Fresh *Salix ledermannii* leaves were obtained from a farm in Mangu Halle, Mangu Local Government Area (Latitude: 9° 30' 59.99" N and Longitude: 9° 05' 60.00" E) in Plateau State, Nigeria, in March 2023. After identifying the plant samples, a specimen was deposited in the herbarium (voucher number JUHN21000415). After drying the samples, they were crushed using mortar and pestle into fine powder, and extraction of the plant compounds was done by cold maceration where 1 part of the powdered leaves was mixed with 10 parts absolute ethanol (99.97 % concentration).[14] 50 g of the powdered leaves were weighed into an extraction flask containing 500 ml absolute ethanol and allowed to extract for 3 days with constant agitation at room temperature.

Phytochemical determination and GC-MS analysis

Phytochemical analysis of *S. ledermannii* was conducted using standardised procedures[14] to detect the presence of bioactive compounds in the plant, such as saponins, alkaloids, tannins, carbohydrates, flavonoids, steroids, cardiac glycosides, anthraquinones, and terpenoids in the extract.[15] GC-MS Analysis was carried out following standard procedures as earlier described[16] and analysed using Mass Hunter software.

Animal use and ethical consideration

Male and female Swiss albino mice weighing between 15 and 30 g were obtained from the Animal House, University of Jos, Jos, Nigeria, and used as models for the study. The test animals were housed in standard conditions and fed standard livestock feed (Super Starter) obtained from Grand Cereals and a local feed bought from the University's Animal House.

Ethical approval was obtained from the Institutional Animal Care and Use Committee (IAACUUC), University of Jos, Nigeria, with certificate number REF/UJ/FPS/PCL/AEU/2 (Appendix 1).

Acute toxicity test

The method of Lorke[17] was adopted for the acute toxicity study. The method involved two phases of different treatment concentrations: Phase 1 (100, 500, and 1000 mg/kg) and Phase 2 (1600, 2900, and 5000 mg/kg).

Parasite inoculation

Plasmodium berghei berghei (N.K. 65 strain) infected mice were obtained from the Animal House, University of Jos, Nigeria. Following the method used previously by Dawet *et al*.[18], the animals were inoculated with the parasites intraperitoneally using standard inoculums (1.2×10^7) of *P. berghei*-infected erythrocytes on day zero for suppressive and curative tests and 72 hours later for prophylactic tests after which the inoculation site was disinfected using an alcohol-based swab.

In vivo antimalarial activities of Salix ledermannii ethanol crude leaves extract

For the *in vivo* test, 75 mice were inoculated with 0.2 ml diluted blood containing 1.2×10^7 parasitised red blood cells for the suppressive, curative, and prophylactic test. After that, an extract of *S. ledermannii* was administered to the mice at doses of 100, 300, and 500 mg/kg, with the negative control group receiving 0.2 ml/kg of distilled water (Dw) and 5 mg/kg chloroquine for the positive control via oral administration. Each group had five replicates.

Suppressive Antimalarial Activity (early malaria infection)

The method described by Dawet and Stephen[19] assessed activities with suppressive effects lasting 4 days. On the first day (D0), twenty-five (25) albino mice of both sexes were first administered 0.2 ml solution containing 1.2×10^7 *P. berghei* intraperitoneally. Some 2–4 hours after inoculation, oral doses of the extract (100, 300, and 500 mg/kg) were administered to the test group. The reference drug group received 5 mg/kg chloroquine, while the negative control group was administered a placebo treatment of 0.2 ml/kg distilled water. Each group had 5 replicates. Treatment was administered daily for three consecutive days. On the fourth day (Day 4), after preparation and Giemsa staining, blood smears made from each mouse were examined microscopically under oil immersion at × 100 objective using a light microscope. Parasitaemia level was determined using equation 1, while suppression of the parasitemia was determined using equation 2. The values were recorded in percentage for each group. Parasitemia level in the control group administered distilled water (Dw) was compared against that of the treatment group.

$$\%\ parasitemia = \frac{number\ of\ parasitized\ red\ blood\ cells}{Total\ red\ blood\ cells\ count} \times 100 \quad (1)$$

Equation 2 below calculates the average percentage suppression for the extract dose administered compared to the control.

$$\%\ suppression = \frac{\%\ parasitaemia\ in\ control - \%\ parasitaemia\ in\ test\ group}{\%\ parasitaemia\ in\ control} \times 100 \quad (2)$$

Curative Antimalarial Activity (established malaria infection)

Twenty-five (25) of the experimental animals (mice) were chosen. Each received an intraperitoneal (i.p.) inoculation of 0.2 ml of 1×10^6 *Plasmodium berghei* parasites and, after that, divided into five groups containing five mice each. After Seventy-two post-inoculation, the experimental groups received oral doses of the extract of 100, 300, and 500 mg/kg/day, respectively. 5 mg/kg of chloroquine was given to the reference drug group, whereas the negative control group received a placebo treatment of 0.2 ml/kg distilled water. The treatments were administered consecutively for 7 days, and blood samples were taken daily from the tail veins, smeared onto slides, and the stained preparations were microscopically examined to determine the level of parasitaemia. On the seventh day after infection, the percentage parasitemia inhibition was calculated using the following equation: 3.[20]

$$\%\ inhibition = \frac{\%\ parasitaemia\ (Initial) - \%\ parasitaemia\ (Final)}{\%\ parasitaemia\ (Initial)} \times 100 \quad (3)$$

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Prophylactic Antimalarial Assay (residual malaria infection)
The prophylactic test was based on the previous method used by Dawet *et al*.[17] The mice were assigned rando mly into six groups of five mice per group. The mice received the following oral administrations: 100, 300, and 500 mg/kg/day of the extract; pyrimethamine (reference drug) at 1.2 mg/kg/day; and 0.2 ml of distilled water for the control group. Each group had five replicates in which treatment was administered for three consecutive days (D0-D2). On day four (D3), they were inoculated with 1×10^6 *P. berghei*. The mice were monitored for an additional three days, after which blood samples were collected on day seven (D7), and smears were prepared for further examination. The level of parasitaemia inhibition was then calculated and expressed as a percentage.

Haematological parameters
Examination of the blood parameters was carried out according to Kabiru *et al*.[21] Blood samples of each mouse were collected at the final stage of the curative test, and PCV was determined using heparinised microhematocrit capillary tubes and a centrifuge for five (5) minutes. Haemoglobin (Hb) level was also determined.

Statistical analysis
Data analysis was done using some statistics in R Console (R-2.15.2). A one-way ANOVA test followed by Turkey's post hoc test was used to analyse the data for statistical significance. Results were considered significant at $p<0.05$.

Results and Discussion

The percentage extraction yield of the ethanol extract of *S. ledermannii* leaf was 33.98 % (16.99 g). Phytochemical results revealed that the bioactive compounds include alkaloids, phenolics, terpenes, saponins, anthraquinones, steroids, and tannins. Furthermore, the GC-MS screening of the extract revealed forty-three (43) known compounds (Figure 1), with benzoic acid (27.21%), phenol (15.22%), *β*-D-Glucopyranose (12.29%), Salicylalcohol (11.88%), 1-Butanol, 3-methyl (6.93%), Catechol (6.35%) and -ethyl-5,6-dihydo-2H-pyran-2-one (5.48%) having the highest concentrations as presented in Table 1. The bioactive compounds in *S. ledermannii* are widely distributed and have been proven to possess medicinal properties such as antimicrobial properties and several important physiological effects in animals.[21] Suharsanti *et al*. [22] have also documented various compounds isolated from cucumber using different extraction methods, including chromatographic techniques. Previous studies have indicated these compounds' anti-microbial, anti-inflammatory, anti-diabetic, anti-atherosclerotic, and anti-carcinogenic properties.[23] A study by Falodun *et al*.[24] demonstrated several compounds' antimalarial and antibacterial potentials in *Persea americana* seed. Although fewer compounds than those reported in this study were observed, the results generally revealed that plants contain invaluable compounds that could serve man's needs, which could be harnessed and used in pharmaceutical industries.

The acute toxicity screening of *S. ledermannii* extract shows no mortality in any groups of the mice after 48 hours of administration, even though an LD_{50} value above 5000 mg/kg was found for the extract. The experimental mice had no changes in the skin, eye mucus membranes, trembling, behavioural patterns, sleep, diarrhoea, falling of fur, or coma. Furthermore, no significant variations were observed in body weight. This implies that *S. ledermannii* is safe for consumption and does not affect the model animals' health.

In the suppressive test, the ethanol extract of *S. ledermannii* exhibited significant chemo-suppression at 100 mg and 300 mg/kg, 44.38 % and 46.75 %, respectively, as shown in Table 2.

Also, in the curative assay, the percentage inhibition of parasitaemia ranged from 89.0 to 82.7%, as shown in Table 3. The highest percentage of inhibition was recorded in the 5 mg/kg-chloroquine-treated group, while the lowest was recorded in an untreated group (control). 5 mg/kg-chloroquine and 300 mg/kg treated group significantly inhibited ($p<.05$) parasitaemia compared to the control.

Similarly, the percentage inhibition of parasitaemia in the prophylactic tests ranged from 14.8-21.4%. The highest percentage of inhibition was recorded in the 500 mg/kg group, while the lowest was recorded in the untreated group (control). *S. ledermannii* ethanol extract showed no significant inhibition ($p>0.05$) of parasitaemia as against the control in the prophylactic test (Table 4). The findings revealed that *S. ledermannii* extract possesses significant anti-plasmodial activity, as observed in the chemo-suppression. Each treatment dose exhibited an increasing rate of chemo-suppression, although a slight reduction in effect was observed with the 500 mg/kg treatment (34.91%). The highest anti-plasmodial suppression (46.75%) recorded using the treatment dose of 300 mg/kg was much lower compared to that reported by Dawet *et al*.[17] in their study, where 90.70% suppression was recorded. In another study, Muhaimin *et al*. [25] recorded suppressions of over 95% using extracts of *Sonneratia alba,* which was very high compared to this study. However, the fact that in all the studies done, the differences observed were statistically significant ($p<0.05$) implies that the compounds in the plants exhibit significant effects on the parasites, and their effects are concentration-dependent. Similar to this study, Muhaimin *et al*.[25] observed that the activity recorded from the plant could be due to the presence of the various bioactive compounds contained in the plant. This research recorded a gradual decrease in parasitemia level, which is, however, similar to the report by Haruna *et al*.[26] on the antimalarial activity of methanol root extract of *Securidaca longepedunculata* on *Plasmodium berghei*. The suppressive effect of 46.75% observed in this study is also lower than the 79.50% by Alli *et al*.[27] and 80% by Bantie *et al*.[28]

In another study, Abosi and Raseroka[29] investigated the antimalarial activity of *Vernonia amygdalina* leaf extract on *P. berghei* and reported 67 % suppression of parasitaemia in the four-day test[30]. The crude leaf extract also exerted a significant curative effect, as shown in the 300 mg/kg (14.2%) treated group, which is likely as a result of the bioactive compounds identified in the extract, which has been reported to possess antiplasmodial activities.[31]. These plants have been shown to act either by causing red blood cell oxidation[32] or inhibiting protein synthesis[33] depending on the compounds they contain. The ethanol leaf extract of *S. ledermannii* demonstrated significant antimalarial activity, with a dose of 500 mg/kg showed a higher repository activity (21.4% inhibition) than pyrimethamine (16.2% inhibition). This finding agrees with Akuodor *et al*. 34, who documented the repository activity of *Vernonia amygdalina*. Similar to our finding, Oghogho *et al*. [31] recorded a significant ($p<0.05$) antimalarial activity on *P. berghei*-infected mice using *Beta vulgaris* extract. Suggesting that the extract might have attacked the young Sporozoites, thereby inhibiting their growth or killing them as in the case of the standard drug (Chloroquine).

This study also reported the effects of *S. ledermannii* extract on haematological parameters *of P. berghei*-infected mice in suppressive, Rane's curative, and prophylactic antimalarial models.

Hemoglobin content ranged from 11.52±1.19-15.6±51.10 g/dl, 10.68±0.72-11.54±0.67 g/dl, and 12.86±1.67-14.26±0.56 g/dl in suppressive, curative and prophylactic antimalarial model, respectively. PCV ranged from 34.2±3.42-44.2±0.84%, 32.00±2.12-34.60±2.07%, and 39.20±3.80-42.80±1.79 % in the suppressive, curative, and prophylactic antimalarial model, respectively. The PCV of the CQ-5 mg/kg treated group was significantly higher ($p<.05$) compared to other groups in the suppressive antimalarial model, as shown in Table 5. Complete blood is used to gain valuable insights into the haematological status of a disease condition.[35] Anaemia, which is one of the manifestations of malaria infection, is assessed by determining the packed cell volume (PCV), haemoglobin (Hb), as well as red blood cell (RBC) count[36] in malaria patients. In this study, it was found that there were some variations in the haematological parameters of *P. berghei*-infected mice treated with *S. ledermannii* leaf extracts. The suppressive antimalarial model showed a significant increase in Packed Cell Volume (PCV) levels in mice treated with Chloroquine (CQ) but not in the curative and prophylactic model. This finding in the suppressive antimalarial model agrees with Adebayo *et al*.[37] Changes in the Hb level of mice treated with *S. ledermannii* crude leaf extracts were not significant in the suppressive, curative, and prophylactic antimalarial model, and this finding agrees with Eze (2019).[38]

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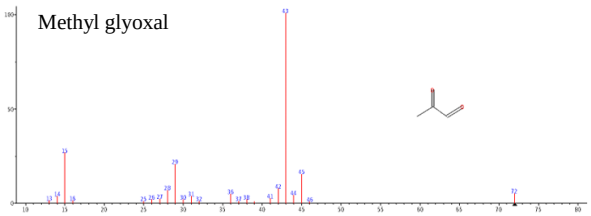
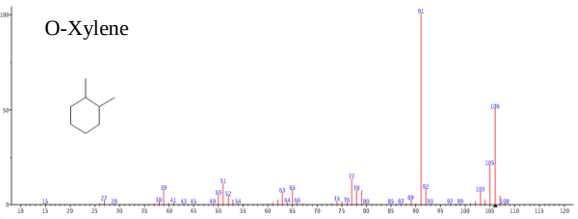
Methyl glyoxal



O-Xylene



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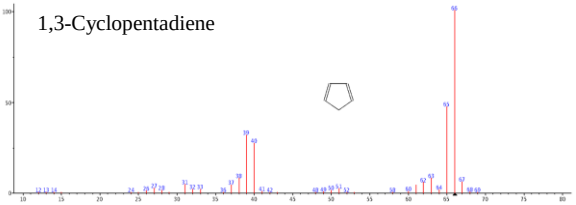

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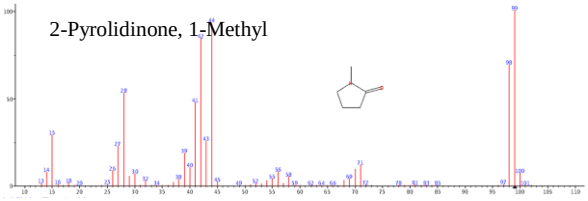

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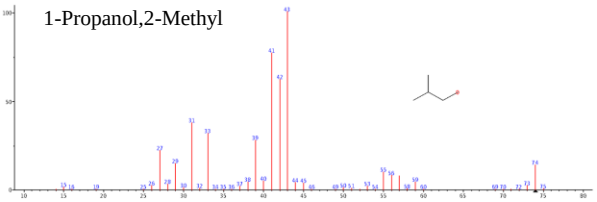

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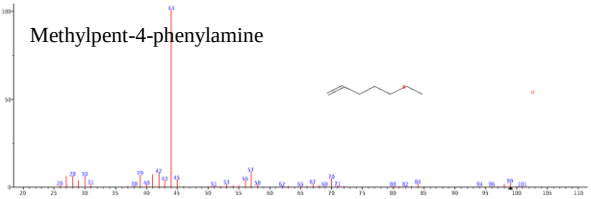

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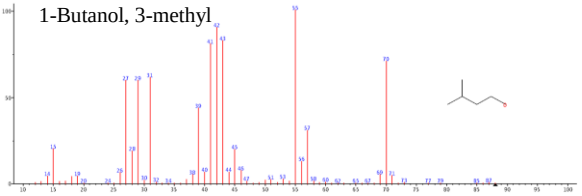

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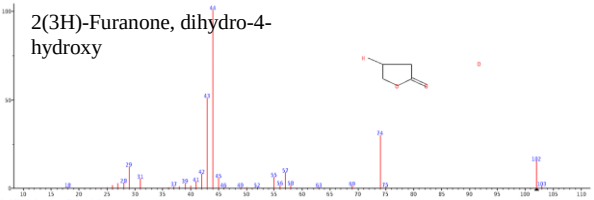

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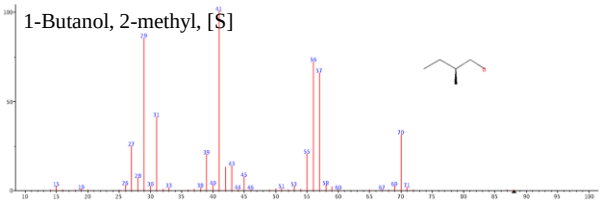

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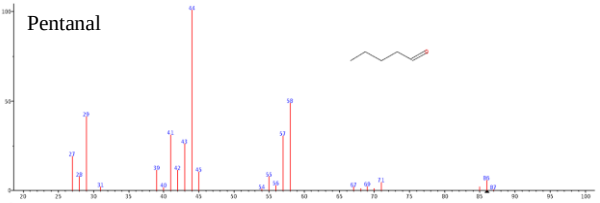

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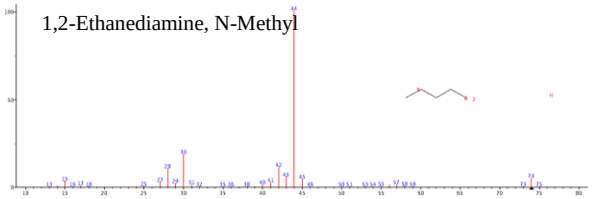

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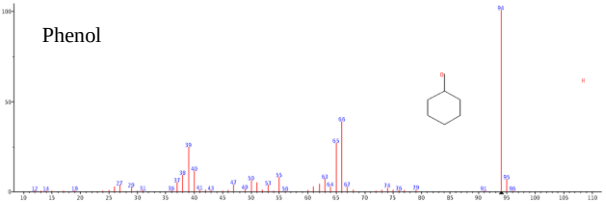

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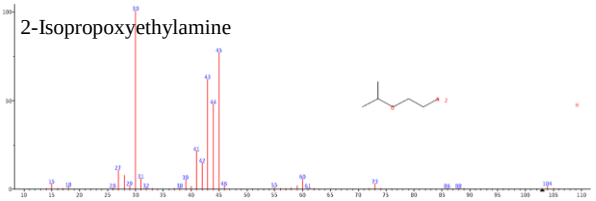

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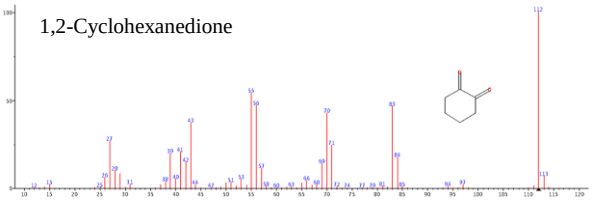

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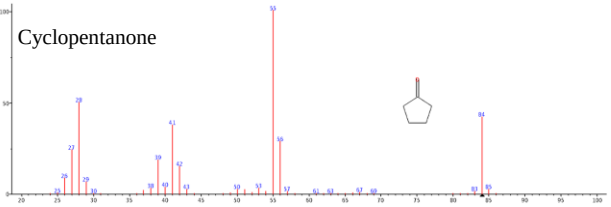

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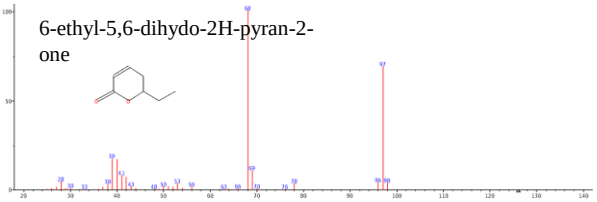

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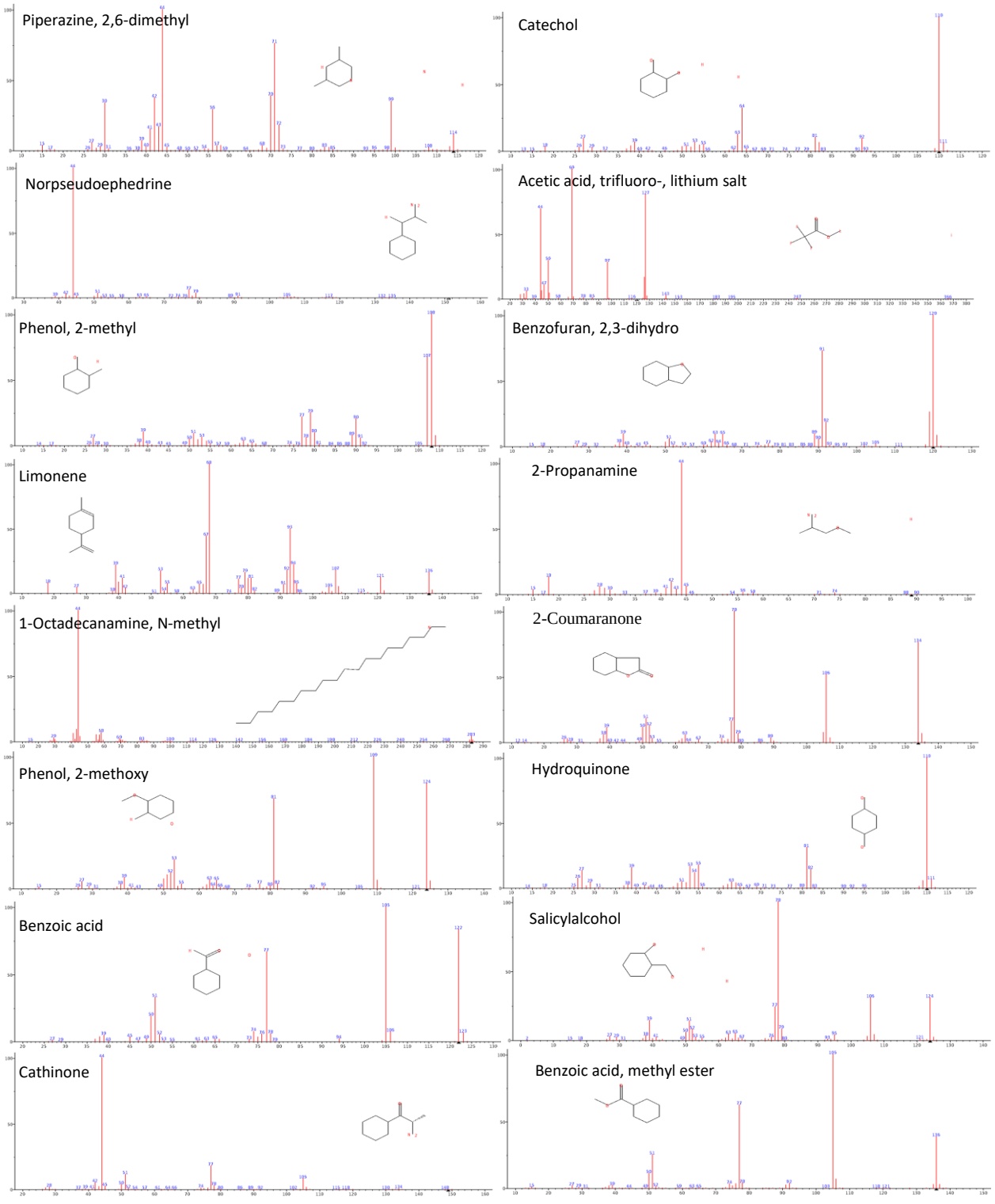

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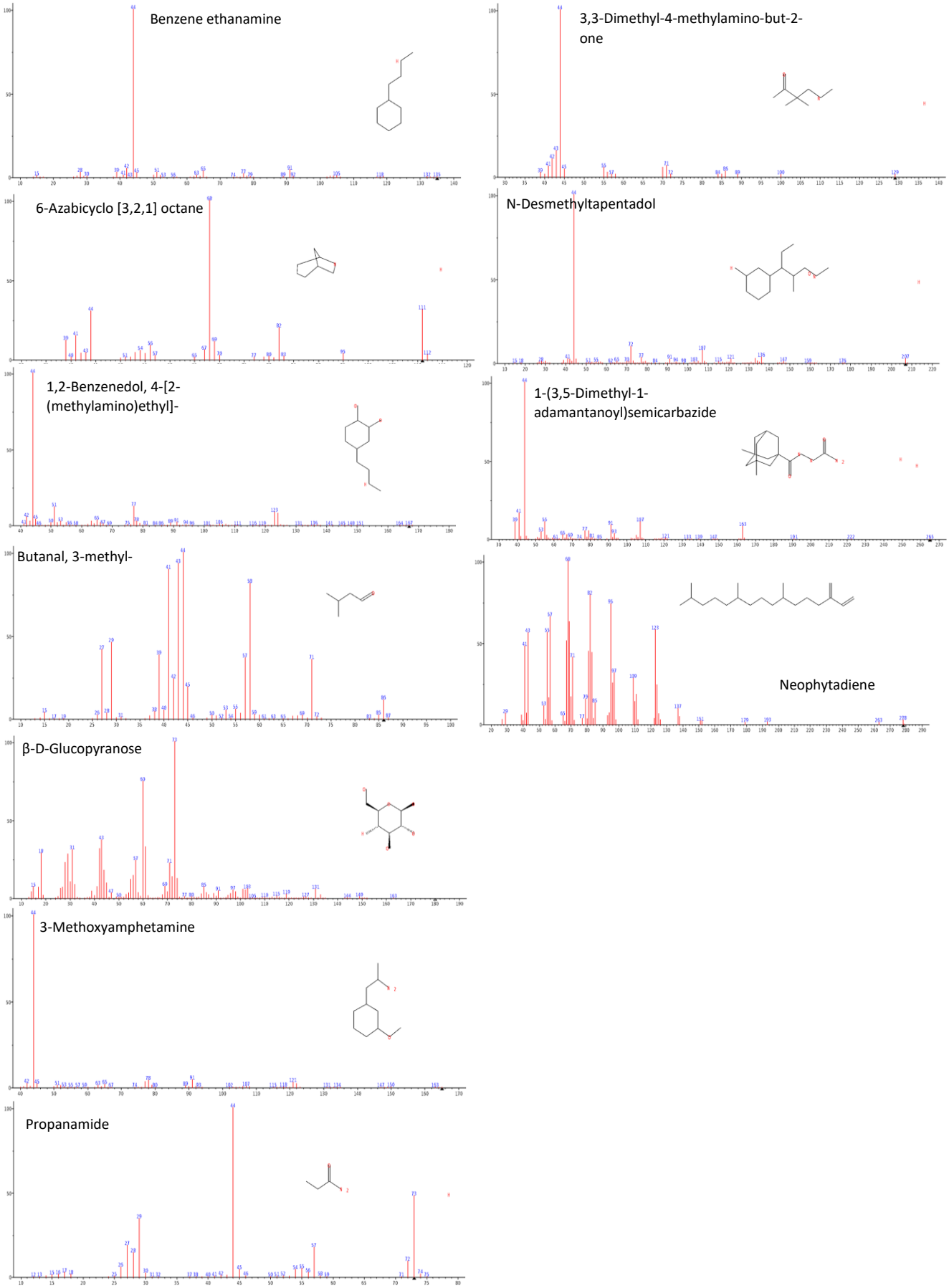

Figure 1: Mass Spectra of Identified Compounds from *Salix ledermannii Ethanol Extracts*

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Table 1: Major bioactive compounds compounds identified from ethanol leaf extract of *S. ledermannii*

No	R.T.	Names of Compounds	Molecular Formula	MW(g/mol)	Peak Area %
1	4.617	Methyl glyoxal	$C_3H_4O_2$	72.06	0.96
2	5.449	1,3-Cyclopentadiene	C_5H_6	66.1	0.3
3	6.746	1-Propanol, 2-methyl	$C_5H_{13}NO$	103.16	0.92
4	9.047	1-Butanol, 3-methyl	$C_6H_{15}NO$	117.19	6.93
5	9.151	1-Butanol, 2-methyl, [S]	$C_5H_{12}O$	88.1	1.56
6	9.313	1,2-Ethanediamine	$C_7H_{18}N_2$	130	0.58
7	9.653	2-Isopropoxyethylamine	$C_5H_{13}NO$	103.16	0.81
8	10.171	Cyclopentanone	C_5H_8O	84.12	2.4
9	12.578	O-Xylene	C_8H_{10}	106.16	0.56
10	13.249	2-Pyrolidinone	C_5H_9NO	99.13	0.62
11	13.41	Methylpent-4-phenylamine	$C_6H_{13}N$	99.17	0.39
12	14.081	2(3H)-Furanone	$C_4H_4O_2$	84.07	0.76
13	14.246	Pentanal	$C_5H_{10}O$	86.13	0.76
14	14.564	Phenol	C_6H_6O	94.11	15.22
15	15.167	1,2-Cyclohexanedione	$C_6H_8O_2$	112.13	3.24
16	15.535	6-ethyl-5,6-dihydo-2H-pyran-2-one	$C_7H_{10}O_2$	126.15	5.48
17	15.677	Piperazine	$C_4H_{10}N_2$	86.14	1.11
18	16.166	Norpseudoephedrine	$C_9H_{13}NO$	151.21	0.41
19	16.281	Phenol, 2-methyl-	C_7H_8O	108.14	2.15
20	16.396	Limonene	$C_{10}H_{16}$	136.23	0.87
21	16.935	1-Octadecanamine	$C_{18}H_{39}N$	69.5	0.53
22	17.196	Phenol, 2-methoxy-	$C_7H_8O_2$	124.14	0.13
23	18.568	Benzoic acid	$C_7H_6O_2$	122.12	27.21
24	18.736	Cathinone	$C_9H_{11}NO$	149.19	1.01
25	19.015	Catechol	$C_6H_6O_2$	119.11	6.35
26	19.335	Acetic acid, trifluoro-, lithium salt	$C_2F_3LiO_2$	120	2.12
27	19.496	Benzofuran, 2,3-dihydro	$C_{15}H_{14}O$	210.27	2.54
28	19.736	2-Propanamine	C_3H_9N	59.11	0.71
29	19.965	2-Coumaranone	$C_8H_6O_2$	134.13	1.3
30	20.178	Hydroquinone	$C_6H_4(OH)_2$	110.11	0.96
31	20.42	Salicylalcohol	$C_7H_8O_2$	124.11	11.88
32	21.603	Benzoic acid, methyl ester	$C_8H_8O_2$	136.15	0.74
33	21.788	Benzene ethanamine	$C_{22}H_{22}FNO_3$	367.4	0.43
34	22.038	6-Azabicyclo[3,2,1]octane	$C_{10}H_{19}N$	153.26	1.46
35	22.773	1,2-Benzenedol, 4-[2-(methylamino)ethyl]-	$C_9H_{14}N_2$	150.22	0.59
36	23.094	Butanal, 3-methyl-	$C_{10}H_{18}O_3$	186.25	1.2
37	23.94	β-D-Glucopyranose	$C_6H_{12}O_6$	180.16	12.29
38	24.979	3-Methoxyamphetamine	$C_{10}H_{15}NO$	165.23	0.41
39	25.078	Propanamide	C_3H_7NO	73.09	0.69
40	25.727	3,3-Dimethyl-4-methylamino-but-2-one	$C_7H_{15}NO$	129.199	1.46
41	28.042	N-Desmethyltapentadol	$C_{13}H_{21}NO$	207.31	1.08
42	28.195	1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	$C_{14}H_{23}N_3O_2$	265.35	2.19
43	30.376	Neophytadiene	$C_{20}H_{38}$	278.5	2.27

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Table 2: Average % Suppression of Parasitaemia of mice infected with *P. berghei* and treated with ethanol extract of *S. ledermannii* (Suppressive Test)

Groups	%Parasitaemia	%Suppression
C1 Dw-0.2 ml	67.60±13.90	--
C2 CQ-5 mg/kg	43.80±8.20	35.21
100 mg/kg	37.60±8.30*	44.38
300 mg/kg	36.00±4.53*	46.75
500 mg/kg	44.00±6.52	34.91

*p<0.05 compared to control

Data are mean ± standard deviation (SD) (n = 5); *p<0.05 as compared with control group; % suppression of parasitemia = (% parasitemia in control − % parasitemia in treated group) / % parasitemia in control) × 100. CQ= Chloroquine; Dw= Distilled water

Table 3: % Inhibition of Parasitaemia in mice infected with *P. berghei* and treated with extract of *S. ledermannii* in Curative Test

	Parasitemia(M±SD)		
Concentration	Initial (D5)	Final (D7)	%Inhibition
C1 Dw-0.2 ml	37.60±5.94	71.00±8.94	-89
C2 CQ-5 mg/kg	31.20±10.33	57.00±4.47	82.7*
100 mg/kg	32.00±15.25	51.40±30.56	-60.6
300 mg/kg	40.80±17.51	35.00±10.00	14.2*
500 mg/kg	35.40±17.95	39.80±10.31	-12.4

Values are mean ± standard deviation (S.D.) (n = 5); *p<0.05 as compared with the control group; % inhibition of parasitaemia = (% parasitaemia on day 5 − % parasitemia on day 7/% parasitaemia on day 5) × 100. CQ= Chloroquine; Dw= Distilled water

Table 4: % Inhibition of Parasitaemia of mice infected with *P. berghei* and treated with extract of *S. ledermannii* in Prophylactic Test

	Parasitaemia(M±SD)		
Groups	Initial (D 6)	Final (D 8)	% Inhibition
C1 Dw-0.2 ml	66.20±13.31	56.4±6.36	14.8
C2 PM-1.2 mg/kg	66.00±16.73	55.3±7.53	16.2
100 mg/kg	67.60±10.89	57.25±6.21	15.3
300 mg/kg	58.80±6.49	52.6±3.01	10.5
500 mg/kg	48.60±9.32	38.2±5.53	21.4

Values are mean ± standard deviation (SD) (n = 5); % inhibition of parasitaemia = (% parasitaemia on day 6 − % parasitaemia on day 8 / % parasitaemia on day 6) × 100; CQ= Chloroquine; Dw= Distilled water

Table 5: Effect of *S. ledermannii* Extract on haematological parameters of P. *berghei*-infected Mice in Suppressive, Rane's Curative, and Prophylactic Antimalarial Model

	HB(g/dl)			PCV (%)		
Groups	Suppressive	Curative	Prophylactic	Suppressive	Curative	Prophylactic
Control (Dw)	11.52±1.19	11.20±0.70	14.00±0.74	34.2±3.42	33.62±2.07	42.00±2.24
CQ-5 mg/kg	14.66±0.35	10.68±0.72	12.86±1.67	44.2±0.84*	32.00±2.12	39.20±3.80
100 mg/kg	11.74±0.43	11.54±0.67	14.20±0.77	35.20±1.30#	34.60±2.07	41.60±2.07
300 mg/kg	11.94±1.01	10.86±0.38	13.12±2.20	36.4±2.61#	32.60±1.14	39.40±6.54
500 mg/kg	15.6±51.10	10.70±0.46	14.26±0.56	38.2±2.60#	33.40±2.30	42.80±1.79

Data are mean ± standard deviation (S.D.) (n = 5); *p<0.05 and #p<0.05 as compared with control and CQ-5 mg/kg groups, respectively. CQ= Chloroquine; Dw= Distilled water.

Conclusion

This study has shown that crude ethanol leave extract of *S. ledermannii* exhibits significant efficacy against *P. berghei* infection as seen in the suppressive test (at 100 and 300 mg/kg), curative (at 300 mg/kg) and prophylactic (at 500 mg/kg) model. *S. ledermannii* poses no adverse effect on PCV and Hb levels of treated mice. There is a need for further study on the components of *S. ledermannii* through isolation and

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characterisation of the active ingredient to ascertain which is the most effective in malaria treatment.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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