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In vivo Antimalarial and GC-MS Studies of *Pennisetum purpureum* Leaf Extract and Fractions

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ABSTRACT

Pennisetum purpureum (elephant grass) is a plant used in ethnomedicine for malaria treatment. The present study was aimed at investigating the antimalarial potential of *P. purpureum* leaf and to identify its phytoconstituents. The leaves were extracted with methanol to afford the crude extract which was successively partitioned between water and *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol to afford the fractions. The acute toxicity study was done in mice. Mice were infected with *Plasmodium berghei* and then treated (p.o.) with the crude extract in the curative and suppressive antimalarial models at three doses (100, 200 and 400 mg/kg). Another set of infected mice was also treated orally with 200 mg/kg of each of the fractions in a suppressive model. The reference drug used for both models was artemeter/lumefantrine (7 mg/kg A/L). The most active fraction, the *n*-hexane fraction, was subjected to further analysis by GC-MS. The crude extract lethal dose (LD_{50}) was established as 1702.94 mg/kg. The crude extract showed a plasmodial inhibitory activity in the range of 46.20 to 59.90% in the curative model. Both the extract and fractions displayed chemo-suppressive activity ($p<0.05$) in the range of 66.90 to 96.50%. The A/L produced ($p<0.05$) 69.00% inhibitory and 95.20% chemo-suppressive activities. The results of GC-MS showed the presence of 21 compounds. It was concluded that the extract and fractions of *P. purpureum* displayed strong antimalarial activity in both models which provides justification for the use of the plant in traditional medicine for malarial treatment.

Keywords: Antimalaria models, GC-MS analysis, Malaria, *Pennisetum purpureum, Plasmodium berghei.*

Introduction

Malaria is a deadly infectious disease which occurs globally but with higher impact in the tropical and subtropical regions.[1] The parasitic disease is transmitted by the female Anopheles mosquito which is the vector of the most important causative organism, *Plasmodium falciparum*. Current estimate shows the incidence of 221 million new cases and 625,000 deaths related to malaria in the year 2020.[2] The disease negatively impacts the economy and productivity of the affected people.[3] One of the greatest concern in the use of the currently available antimalarial drugs is the drug resistance of the causative organisms, including the artemisinin-based combination therapies (ACT).[4] New antimalarial agents are needed to overcome the issue of resistance as well as other limitations of the existing drugs such as their adverse effects. Medicinal plants have been used over the years to cure malaria. One of such plants which have been documented is *Pennisetum purpureum* Schumach. (Poaceae). *P.purpureum* (elephant grass) is a perennial tropical grass that is used for the treatment of various diseases including haemorrhoids and malaria.[5,6] The phytoconstituents of the plant include flavonoids, tannins, alkaloids and saponins.[7] Herein, the antimalarial potentials as well as the phytoconstituents of *P. purpureum* leaf extract and fractions are reported.

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Materials and Methods

Plant materials collection and preparation

The leaves of *P. purpureum* Schumach were collected in July, 2017 from Edem Ani (6.8698° N, 7.3571°E) in Nsukka, Nigeria. Identification (voucher: PCG/UNN/0082) was done at the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka where the specimen was deposited. The materials were air-dried for two weeks, ground into a fine powder with a mechanical grinder (Manesty, England) and stored in a refrigerator at 4°C for future use.

Extraction and fractionation

Accurately weighed (700 g) quantity of the powder was macerated in 3.5 L of 95% methanol for three days with occasional agitation. This was filtered and then concentrated using a rotary evaporator. This afforded the crude extract (PPE). A certain quantity (25.00 g) of PPE was successively fractionated by partitioning between water and *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The respective fractions were coded respectively as PPH, PPD, PPEA, PPB and PPW.

Calculation of yield value

Calculation of the yield (%) of the crude extract (PPE) was based on the weight (700 g) of the dry powder taken for extraction. The yield (%) of the fractions was calculated based on the weight (25.0 g) of the extract (PPE) taken for fractionation according to Eq. 1 and Eq. 2:[8]

$$\%Yield\ of\ extract = \frac{weight\ of\ extract}{weight\ of\ plant\ powder} \times 100\% \text{ …. Eq. 1.}$$

$$\%Yield\ of\ fraction = \frac{weight\ of\ fraction}{weight\ of\ extract} \times 100\% \text{ …….. Eq. 2.}$$

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Animals
The animals used were adult Swiss albino mice of both sexes having mean body weight of 18.5 ± 2.2 g. The animals were maintained at room temperature of 22±3ºC, relative humidity of 40–50% with 12 h light/ 12 h dark cycle. The study was done following the International Council for Laboratory Animal Science (ICLAS) guidelines as well as the institutional animal ethical committee (approval number: FPSRE/UNN/21/0001).

Acute toxicity studies
An acute toxicity test was conducted in two stages as described originally by Lorke[9] and also reported recently by Ibrahim *et al*.[10] Nine mice were employed in the first stage; these were divided into three groups (n = 3). The PPE was given at the three doses of 10, 100 and 1000 mg/kg. All extract administrations were done orally (p.o.). After the extract administration, the mice were observed for 24 h for mortality and signs of toxicity. The mice were observed for a further14 days to ascertain survival. One animal died in the first stage; hence the dosing for the second stage was 600, 1000, 1600 and 2900 mg/kg.[9] The lethal dose (LD_{50}) was calculated following Eq. 3:

$$LD_{50} = \sqrt{minimal\ lethal\ dose \times maximal\ survival\ dose}$$ .. Eq. 3

Inoculation of Parasites
Inoculum was prepared from the parasitized (with *P. berghei)* blood collected from the donor mice. Blood was serially diluted with normal saline solution to make a suspension so that 0.2 mL contained approximately$1x10^7$ infected RBC (red blood cells).[11]

Curative antimalarial test
The crude extract was assessed for its malarial curative potential following the method previously described by Bantie *et al*.[11] as well as Mzena *et al*.[12] On Day 0, mice were administered (*i.p.*) with 0.2 mL of standard inoculum. On Day 3 (72 h after parasite inoculation), the parasitized mice were divided randomly into five (5) groups each having five (5) mice and were treated (*p.o.*) once daily for five days. The animals in the groups received as follows:
Groups 1: 100 mg/kg of PPE
Groups 2: 200 mg/kg of PPE
Groups 3: 400 mg/kg of PPE
Groups 4: standard drug, 7 mg/kg of artemeter/lumefantrine(A/L).
Group 5: vehicle only (2% Tween 80 administered at the dose of 10 mL/kg)
The doses selected for the extract were based on the results of acute toxicity studies while the doses for the A/L and the vehicle were based on previous reports.[8, 13] Parasitaemia level was determined on Day 3 and then Day 8 from the thin blood film prepared from the tail of each mouse. Three slides were prepared for each mouse and these were examined under a light microscope (Olympus CE). On each slide, three fields were examined to count the RBC. The mean count was taken and the result was used to calculate the %parasitaemia level as shown in the following formula (Eq. 4)

$$\%\ \text{Parasitaemia} = \frac{\text{number of parasitized RBC}}{\text{total number of RBC}} \times 100$$ …Eq. 4.

The inhibition of parasitaemia was calculated using Eq. 5

$$\%\ \text{Inhibition of parasitaemia} = \frac{(\%\ \text{parasitaemia on Day 3} - \%\ \text{parasitaemia on Day 8})}{\%\text{parasitaemia on Day 3}} \times 100$$ …Eq. 5

The mice were sacrificed on Day 8 and the blood samples collected in heparinized capillary tubes for hematological analysis.

The suppressive test
The suppressive test was done for four days using the crude extract and the fractions as reported previously.[11-13] Fifty (50) infected mice were randomly grouped (n = 5) into ten (10) groups. The animals in the groups received as follows:
Groups 1:100 PPE
Groups 2: 200 PPE
Groups 3: 400 PPE
Groups 4: 200 mg/kg PPH
Groups 5: 200 mg/kg PPD
Groups 6: 200 mg/kg PPEA
Groups 7: 200 mg/kg PPB
Groups 8: 200 mg/kg PPW
Groups 9: 7 mg/kg A/L
Group 10 (control): vehicle (10 mL/kg of 2% Tween 80).
Inoculation (0.2 mL of standard inocula, *i.p.*) was done on Day 0 and treatment (p.o) was started three hours (3 h) later. The treatment was given once daily for four consecutive days. After treatment was completed, parasitaemia was determined as described in the curative model by thin blood film studies. Suppression (%) was calculated using Eq. 6. Each mouse was also observed daily for 30 days to determine the mean survival time (MST) as reported.[8]

$$\%\text{Suppression} = \frac{(\text{mean parasitaemia of control group} - \text{mean parasitaemia of treated group})}{\text{mean parasitaemia of control group}} \times 100$$
..Eq. 6

Packed cell volume (PCV) measurement
The PCV was determined by employing a micro-hematocrit reader (Hawksley, Finlab) as reported earlier.[11-13] Percentage (%) change in PCV was calculated using Eq. 7.

$$\%Change\ in\ PCV = \frac{(PCV\ on\ Day\ 8 - PCV\ on\ Day\ 3)}{PCV\ on\ Day\ 3} \times 100$$ …..Eq. 7

GC-MS analysis
GC-MS analysis was done using the most active fraction (*n*-hexane fraction) following previous procedures.[14] The equipment used for the analysis was GCMS-Q2010 Ultra, Shimadzu gas chromatograph.

Statistical Analysis
Data was analyzed using SPSS, version 21.0. Results were expressed as mean ± SEM. Mean comparison with the control was by ANOVA and Dunnet's post hoc test; $p < 0.05$ was considered significant.

Results and Discussion

The results of the extractive yield are presented in Table 1 which shows the yield of the crude extract as 5.36% and the fractions as 2.35% (ethyl acetate fraction) and 48.10% (*n*-hexane fraction).The most abundant fraction was the n-hexane fraction.
In the acute toxicity studies, one of the mice given 1000 mg/kg of PPE in the first stage died but no mortality occurred in the second stage. Thus, the minimal lethal dose was 1000 mg/kg while the maximal survival dose was 2900 mg/kg. The LD_{50} value was therefore calculated as 1702.94 mg/kg.
The present study investigated the antimalarial potentials of *P. purpureum* leaf in two mouse models of malaria, the curative and suppressive models. Administration of the plant extract produced a reduction ($p<0.05$) of parasitaemia levels with chemocurative effect in the range of 42.60 to 59.90% (Table 2). However, the standard drug (A/L) produced a better reduction in parasitaemia level (69.00%) compared to the extract (59.90% at 400 mg/kg which was the highest dose).

Table 1: The yield of the crude extract and fractions of *P. purpureum* leaf

Sample	Yield (g)	Percentage yield (%)[a]
PPE	37.52	5.36
PPH	12.03	48.12
PPD	0.76	3.04
PPEA	0.59	2.36
PPB	1.43	5.72
PPW	3.18	12.72

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In the suppressive model, the crude extract displayed (p<0.05) chemo-suppression (Table 3). The 400 mg/kg dose produced the highest percentage chemo-suppression of 96.50% among the crude extract doses. Similarly, all the fractions produced (p<0.05) chemo-suppressive activity. Among the fractions, the hexane fraction had the highest chemo-suppressive effect of 91.70%, followed by aqueous (87.60%), *n*-butanol (83.00%), ethyl acetate (81.40%) and dichloromethane (66.90%) fractions. Thus, the fraction that produced the least effect was the dichloromethane fraction (PPD); the effect of the A/L was 95.20% (p<0.05). The findings from the present study are in agreement with those of the recent reports on the antimalarial potentials of ethanolic extracts of *P.purpureum* leaf.[15, 16] In addition, other plants in the Poaceae family, including *P. polystachion*[17] and *Cymbopogoncitratus*[18] were also reported to show antiplasmodial potentials. These reports further support the antimalarial potentials of *P. purpureum* since they are of the same family of plants.

PCV results (Table 2) revealed that the PPE as well as the A/L increased the PCV of the mice. PCV gives an indication of the efficacy of test agents in preventing hemolysis due to malaria.[19] In the present study, the improvement in the PCV of the mice suggests a hematopoietic effect which further supports the antimalarial activity of *P. purpureum*. Results of MST in the suppressive model (Table 3) revealed that the *n*-hexane fraction (PPH) prolonged (p<0.05) the life of the animals. Other fractions also had pronounced effect on the MST. Agents which prolong the survival times of malaria-infected animals are considered as possessing antimalarial property.[12] This probably indicates that the *n*-hexane fraction suppresses the parasites and reduces their virulence in mice.[12]

Table 2: Effect of *P. purpureum* extract on parasitaemiaand PCV levels in the curative antimalarial model

Group	Treatment	% Parasitaemia			PCV (%)		
		Day3	Day8	% Inhibition	Day3	Day8	% Change
1	PPE 100mg/kg	68.00 ± 0.94	39.00 ± 3.47*	42.60	28.20 ± 2.72	28.01 ± 3.20	-0.67
2	PPE 200mg/kg	61.40 ± 1.88	31.80 ± 3.89*	48.20	32.60 ± 1.61	37.43 ± 1.43*	14.82
3	PPE 400mg/kg	64.40 ± 4.26	25.80 ± 4.05*	59.90	33.62 ± 0.92	38.80 ± 1.01*	15.41
4	A/L (7mg/kg)	78.80 ± 3.58	24.40 ± 3.02*	69.00	32.64 ± 1.60	39.80 ± 0.66*	21.94
5	Control (infected but untreated)	71.20 ± 2.47	79.60 ± 5.78	-11.80	28.60 ± 1.32	24.03 ± 1.44	-15.98

Data are presented as mean ± SEM; n = 5;*p<0.05significant

Table 3: Effect of *P. purpureum* extract and fractions on parasitaemia level and MST in the suppressive antimalarial model

Group	Treatment	Parasitaemia (%)	Suppression in parasitaemia (%)	MST (days)
1	PPE 100 mg/kg	0.46 ± 0.09*	94.10	16.75 ± 0.85
2	PPE 200 mg/kg	0.50 ± 0.08*	93.20	20.00 ± 2.81
3	PPE 400 mg/kg	0.23 ± 0.07*	96.50	22.00 ± 3.06
4	PPH 200 mg/kg	0.33 ± 0.10*	91.70	23.00 ± 2.57*
5	PPD 200 mg/kg	2.21 ± 0.18*	66.90	13.50 ± 0.87
6	PPEA 200 mg/kg	1.10 ± 0.23*	81.40	20.00 ± 2.81
7	PPB 200 mg/kg	1.08 ± 0.16*	83.00	19.25 ± 1.31
8	PPW 200 mg/kg	0.75 ± 0.14*	87.60	17.80 ± 0.97
9	A/L (7 mg/kg)	0.32 ± 0.80*	95.20	28.00 ± 1.33*
	Control			
10	(Infected but untreated)	10.78 ± 0.63	0	10.80 ± 4.36

Data are presented as mean ± SEM; n = 5; *p<0.05 significant

It was also observed that the *n*-hexane fraction was the most abundant fraction (Table 1) which suggests that high concentrations of the bioactive constituents of the plant are present in the fraction and the biological activity may be attributed to them. The results of GC - MS (Figure 1, Table 4) showed the presence 21 phytoconstituents. The compounds are classified into six major chemo-types. The chemo-types are fatty acids and esters (62.2%), fatty alcohols (21.79%), steroids (4.09%), hydrocarbons (1.03%), ethers (0.85%) and alkaloids (0.06%). The fatty acids and esters as well as fatty alcohols constitute almost 84% of the total constituents. The most abundant peaks based on their peak areas were peak 20 (15-hydroxypentadecanoic acid; 18.35%), peak 11(9-octadecynoic acid, methyl ester; 14.60%) and peak 19 (levo-menthoxyacetic acid; 12.45%). These phytoconstituents could play major roles in the antimalarial activity of the plant. They have been documented for their roles as antimalarial agents in plants. They include long chain fatty acids and their esters[20], alkaloids[21] as well as steroids and terpenoids.[22] These previous reports on the phytoconstituents have given further credence to the antimalarial activity observed in the *P. purpureum*.

Conclusion

The present study showed that *P. purpureum* leaf displayed strong antiplasmodial activity in animal models of malaria. The most bioactive fraction was the *n*-hexane fraction which has fatty acids and esters as the major constituents. These provide justification for the use of the plant in traditional medicine for malarial 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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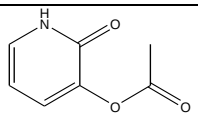
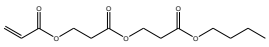
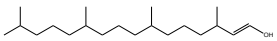
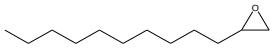
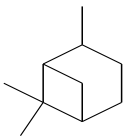
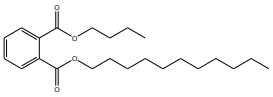
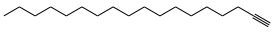
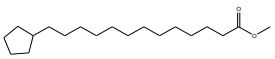
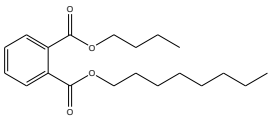
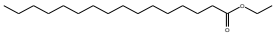
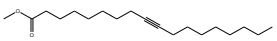
Table 4: Results of GC-MS analysis

P/N	Retention Time (RT) in minutes	Name of Compound	Molecular structure, formular and weight	Class of compound	Peak Area (%)
1	12.767	3-Acetoxy-2(IH)-pyridone	 $C_7H_7NO_3$ (M = 153.14)	Alkaloid	0.06
2	12.943	3,7,11-Trioxo-4,8,12-trioxa-1-hexadecene	 $C_{13}H_{20}O_6$ (M = 272.29)	Fatty ester	0.07
3	15.005	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	 $C_{20}H_{40}O$ (M = 296.53	Fatty alcohol	2.47
4	15.056	Oxirane, decyl-	 $C_{12}H_{24}O$ (184.32)	Fatty ether	0.85
5	15.189	2,6,6-trimethyl- bicyclo[3.1.1]heptane	 $C_{10}H_{18}$ (M = 138.25)	Monoterpene/hydrocarbon	0.45
6	15.253	Phthalic acid, butyl undecyl ester	 $C_{23}H_{36}O_4$ (M = 376.53)	Fatty ester	0.92
7	15.330	1-Octadecyne	 $C_{18}H_{34}$ (M = 250.46)	Aliphatic hydrocarbon	0.58
8	15.652	Cyclopentanetridecanoic acid, methyl ester	 $C_{19}H_{36}O_2$ (M = 296.49)	Fatty ester	2.27
9	15.951	Phthalic acid, butyl octyl ester	 $C_{20}H_{30}O_4$ (M = 334.45)	Fatty ester	2.31
10	16.128	Hexadecanoic acid, ethyl ester	 $C_{18}H_{36}O_2$ (M = 284.48)	Fatty ester	2.73
11	16.844	9-Octadecynoic acid, methyl ester	 $C_{19}H_{34}O_2$ (294.47)	Fatty ester	14.60

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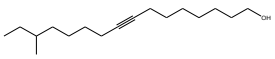
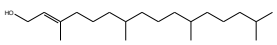
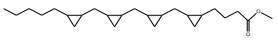
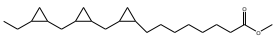
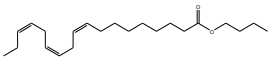
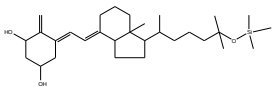
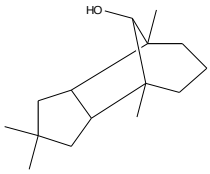
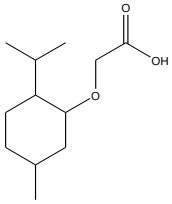
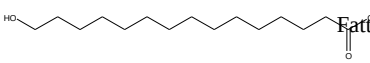
12	16.887	(*R*)-(-)-14-Methyl-8-hexadecyn-1-ol	 $C_{17}H_{32}O$ (M = 252.44)	Fatty alcohol	3.01
13	16.959	Phytol (2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-)	 $C_{20}H_{40}O$ (M = 296.53)	Fatty alcohol	7.24
14	17.037	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	 $C_{25}H_{42}O_2$ (M = 374.32)	Fatty ester	2.58
15	17.282	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	 $C_{22}H_{38}O_2$ (M = 334.45)	Fatty ester	1.54
16	17.329	Butyl 9,12,15-octadecatrienoate	 $C_{22}H_{38}O_2$ (M = 334.54)	Fatty ester	4.38
17	17.473	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3.beta.,5Z,7E)-	 $C_{30}H_{52}O_3Si$ (M = 488.82)	Steroid	6.76
18	18.228	4,8-Methanoazulen-9-ol, decahydro-2,2,4,8-tetramethyl-,	 $C_{15}H_{26}O$ (M = 222.37)	Fatty alcohol	9.07
19	19.699	Levo-menthoxyacetic acid	 $C_{18}H_{32}O_2$ (M = 214.30)	terpenoid acid	12.45
20	19.914	15-Hydroxypentadecanoic acid	 $C_{15}H_{30}O_3$ (258.40)	Fatty acid	18.35
21	20.175	D-Norandrostane-16-methanol, (5α,16β)-		Steroid	7.33

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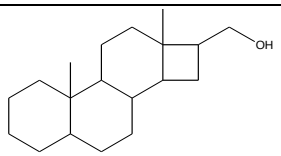

$C_{19}H_{32}O$

(M = 276.46)

P/N = peak number

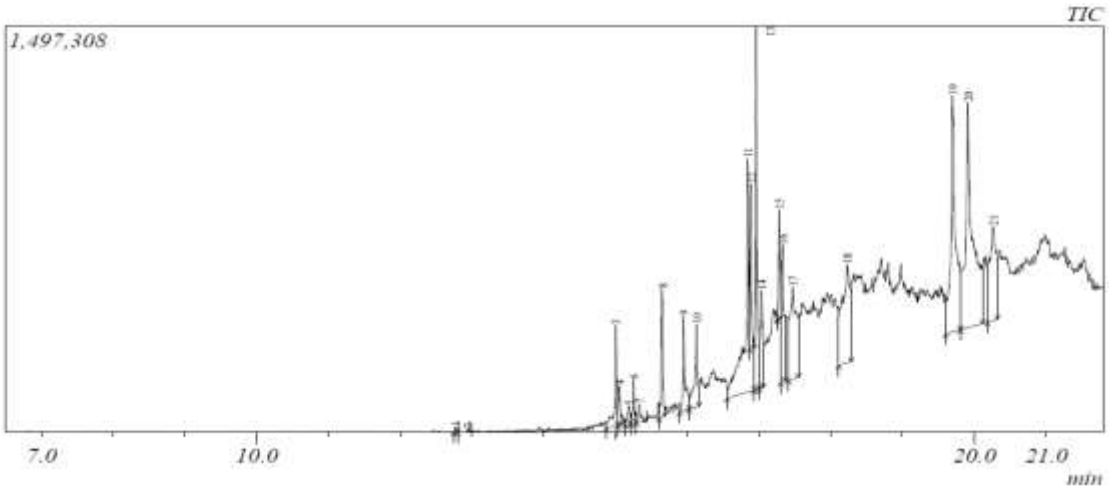

Figure1: GC-MS chromatogram of the hexane fraction of *P. purpureum* leaf extract

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