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Antimalarial potential of leaves crude extract of *Callistemon lanceolatus* D.C.

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Abstract

Plants have been used from ancient times for various purposes like food, shelter and also as a medicine to treat various diseases. The current study was based on an investigation of cheap and herbal drugs against malarial parasite. For the study, chloroform fractions of *Callistemon lanceolatus* of dose concentration 200µl/ml were applied against the malaria parasite. The blood samples (n=67) from male and female of various ages were collected from district Bannu. It was identified that 200µl/ml of chloroform fraction application demonstrated 54.58% inhibition (males=54.70%) of the malarial parasite. The Nivaquine (40 µg/ml) used as positive control exhibited 100% inhibition of malaria parasite. Additionally, distilled water used as negative control (200µl/ml) showed neutral effect. Conclusively, it is found that the chloroform fractions (200µl/ml) of *Callistemon lanceolatus* are effective against malaria disease and may the varied dose concentration be more profound against the disease.

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Introduction

It is reported that 80% of the world population totally depends on plants for their health care and wound healing purpose. However, in developed countries, dependence on surgery and pharmaceutical products is more usual. Nowadays, their attention is diverting towards the natural supplements, rapidly. This motivation of people towards herbs is due to their concern about the harmful effects of drugs, which are prepared from synthetic materials (Khalil *et al.*, 2007).

The genus *Callistemon* (Myrtaceae) has 34 species of beautiful evergreen shrubs and small trees. Most of the *Callistemon* species are common to the more temperate regions of Australia, four species are found in New Caledonia and seven species have been introduced to India as ornamental trees (Kanjilal and Das, 1992). They are generally known as bottle brushes because of their cylindrical brush like flowers similar to the traditional bottle brush. *Callistemon lanceolatus*, also named as *Callistemon citrinus*, is a well- known shrub. Leaves of this plant are used as a tea substitute and have an energizing flavor. Several phenolic compounds of this plant have been identified (Mahmoud *et al.*, 2002b). *Callistemon species* are used for forestry, important oil production, farm tree/windbreak plantings, degraded-land recovery and ornamental horticulture, among other applications (Spencer and Lumley, 1991). In China, *Callistemon* species, chiefly *Callistemon viminalis*, are used in traditional Chinese medicinal drugs for treating hemorrhoids (Ji, 2009). *Callistemon* is also used for the control of weeds (Wheeler, 2005a) and as bioindicators for environmental management (Burchett *et al.*, 2002).

In earlier phytochemical studies, the extraction of *Callistemonlanceolatus* leaves resulted in the isolation of triterpenes (Younes, 1975; Kim *et al.*, 2009), flavonoids (Park *et al.*, 2010), phenolic compounds (Lounasmaa *et al.*, 1977) and tannin derivatives(Marzouk, 2008). Malaria is a disease with more than 200 million population is affected from this infection globally (Cho *et al.*, 2001).

The disease was commonly called ague or marsh fever due to its association with swamps and marshland. For the first time, malaria was discovered by a French Army doctor Charles Louis Alphonse Laveran in 6 November 1880. In the present study, it is believed that the crude leave extracts of the plant has not been screened for anti-malarial activity previously.

Materials and methods

Collection of plant materials

The fresh plant material (leaves) was collected from the Kotka Mir Alam Daud Shah, District Bannu, Khyber Pakhtunkhwa, Pakistan. Material was washed with distilled water and dried under shade at room temperature.

Extraction

Extraction was done by simple maceration. 100 g powder of *Callistemon lanceolatus* was chopped and soaked in 1000 ml of chloroform. The soaked material was shaked thrice a day and lid of the bottle was kept open for few seconds to evaporate fumes produced by solvent shaking.

Then, it was tightly closed. After every 3 days, the material was filtered through a filtration assembly by using a Whatman's filter paper No.1 to avoid impurities and the residue was re-soaked in chloroform solvents for 7 days. At the 7th day, the material was again filtered with Whatman's filter paper No.1. The filtrate was passed through rotary evaporator in order to separate the solvent from gummy extract.

Blood samples collection of malarial patients

The blood samples were collected from 100 malarial patients from district Bannu. The2 ml of blood was collected randomly from each patient of various ages and sexes.

Medium preparation

For the growth and culture of malarial parasite obtained from the sample, culture medium RPMI 1640 (Gibco.USA) was prepared from the stock. The

preparation of RPMI 1640 culture medium involves following steps:

0.3g/30ml of medium RPMI 1640 was dissolved in distilled water.

This culture medium was dropped in the 100 vials of Bijoubottle; each bottle was containing 5 ml of the dissolved medium with supplemented 10% fetal bovine serum (FBS).

Culturing of malarial blood samples

For the growth of malarial parasites, the cultures were maintained in the laboratory using the Candle Jar Method for the human red blood cells (blood type [b]).

The malaria samples were cultured in 67 tubes using RPMI 1640 media. 5 ml RPMI 1640 medium was poured in each tube along with 2ml malarial blood and kept them in incubator for next 72 hours.

Preparation of stock solution from different fractions of plant material

The stock solution was prepared by dissolving 0.25 g of chloroform extract was in 25ml distilled water.

Application of extract on malarial parasites

Theanti-malarial test was performed in 100 tubes. Each test sample was tested with concentration of 10mg/100ml stock solution along with control. 20µL of each test sample was dispensed into each tube containing 100 µL of the infected blood sample. Control consisted of infected blood in culture medium with no test sample i.e., untreated.

The content of each tube was gently mixed and then incubated without agitation for 48 hours at 37°C. After 48 hours, Giemsa-stained blood films on glass slide were prepared from blood sample of each vial. Their numbers of surviving parasites were observed microscopically. Percent inhibition was determined with respect to control samples (Kerharo and Adam, 1974). The percent inhibition was evaluated by applying the formula;

(Number of Plasmodium in the test vial/Number of Plasmodium per control vial × 100) (Mishra *et al.*, 2009).

Statistical analysis

All the data were analyzed by the software SPSS 16 applying Chi-square test.

Result and discussion

Effect of chloroform crude extract (200µl/ml) of Callistemon lanceolatus on the male malarial patients

Chloroform crude extract (200µL/ml) of *Callistemon lanceolatus* was tested against malarial parasites (*Plasmodium vivax* and *Plasmodium falciparum*) present in the blood of male malarial patients.

Table 1. Effect of chloroform crude extract (200µl/ml) of *Callistemon lanceolatus*on the male malarial patients.

Age (Years)	% Inhibition	-Ve control (D.W)	+Ve control (Nivaquine)
1	40	0	100
2	33	0	100
2	50	0	100
3	42	0	100
3	33	0	100
3	38	0	100
4.3	50	0	100
5	40	0	100
5	44	0	100
6	40	0	100
6	32	0	100
6.6	33	0	100
7	40	0	100
7	43	0	100
8	33	0	100

10	50	0	100
12	36	0	100
12	36	0	100
12	33	0	100
12	50	0	100
13	40	0	100
14	43	0	100
14	34	0	100
14	40	0	100
14	37	0	100
14	32	0	100
15	40	0	100
15	50	0	100
15	36	0	100
15	33	0	100
15	38	0	100
15	40	0	100
16	43	0	100
17	50	0	100
17	40	0	100
17	40	0	100
18	33	0	100
19	36	0	100
19	40	0	100
19	43	0	100
20	43	0	100
20	33	0	100
20	50	0	100
20	33	0	100
20	33	0	100
20	43	0	100
21	50	0	100
21	33	0	100
22	43	0	100
22	43	0	100
23	40	0	100
23	33	0	100
23	38	0	100
23	33	0	100
23	50	0	100
25	38	0	100
25	33	0	100
25	33	0	100
27	40	0	100
27	38	0	100
28	33	0	100
29	40	0	100
30	44	0	100
30	33	0	100
30	36	0	100
36	40	0	100
65	33	0	100

D.W= distilled water $X^2 = 227.5208$, $P \leq 0.990$.

The maximum results were recorded in the case of 2, 4.3, 10, 12, 15, 17, 20, 21 and 23 years patients (Table 1, Fig. 1a-c). Similarly, the minimum inhibition was observed (32%) in the case of 6 and 14 years patients. In addition to this, distilled water extract (negative control) caused no inhibition and Nivaquine as positive control (40µg/ml) inhibited the growth of two parasites completely.

Table 2. Effect of chloroform extract of *Callistemon lanceolatus* (200µl/ml) on the female malarial patients.

Age (Years)	% inhibition	-Ve control (D.W)	+Ve control (Nivaquine)
3	33	0	100
3	38	0	100
4	33	0	100
4	50	0	100
6	36	0	100
8	40	0	100
11	40	0	100
12	40	0	100
12	38	0	100
12	33	0	100
12	50	0	100
12	38	0	100
12	33	0	100
13	33	0	100
14	33	0	100
15	33	0	100
15	36	0	100
16	33	0	100
17	43	0	100
18	50	0	100
18	40	0	100
20	38	0	100
20	40	0	100
21	40	0	100
22	40	0	100
22	40	0	100
25	40	0	100
29	38	0	100
31	36	0	100
38	50	0	100
40	36	0	100
45	30	0	100

D.W= distilled water $X^2 = 140.5481$, $P \leq 0.097$.

Table 3. Comparative antimalarial activity of *Callistemon lanceolatus* D.C.

Plant extracts	Part used	Extracts (µg/ml)	Inhibition	Methods	Reference
Callistemon lanceolatus	Leaves	200 µg/ml	50%	Candle jar	Present study
Cassia alata	Leaves	128µg/ml	100%	Candle jar	[19]
Andrographis paniculata	Aerial parts	7.2µg/ml	50%	Candle jar	[20]
Hedyotis corymbosa	Aerial parts	10.8µg/ml	50%	Candle jar	[21]
Bridelia cathartica	Stem	0.05µg/mL	50%	Serial dilutions	[22]
Annona muricata	Leaf	1000 mg/kg	85.61%	Serial dilutions	[23]
Myrtus communis	Aerial parts	35.44 µg/ml	84.8	Candle jar	[24]
Vernonia amygdalina	Leaves	50mg/ml	50%	Candle jar	[25]

Effect of chloroform extract of Callistemon lanceolatus (200µl/ml) on the female malarial patients

The chloroform crude extract (200µL/ml)of *Callistemon lanceolatus*caused maximum inhibition i.e.50% at the age of 4, 12, 18 and 38 years and minimum 30% inhibition at the age of 45 years of female malarial patients (Table 2, Fig.2a & 2b).

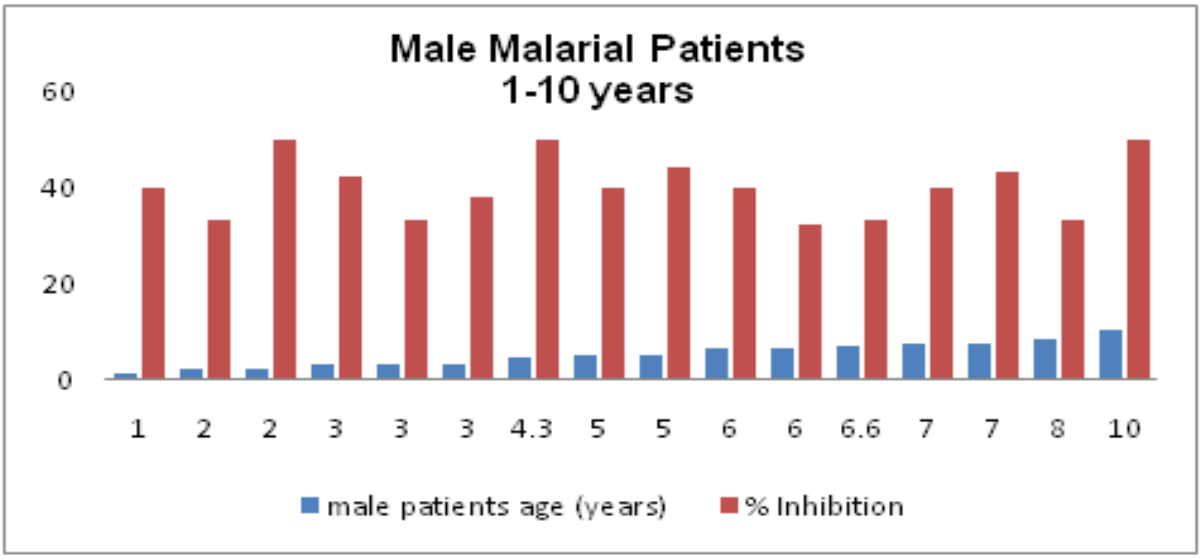

Fig. 1a. Antimalarial effect of chloroform crude extract in male patients (1-10 years).

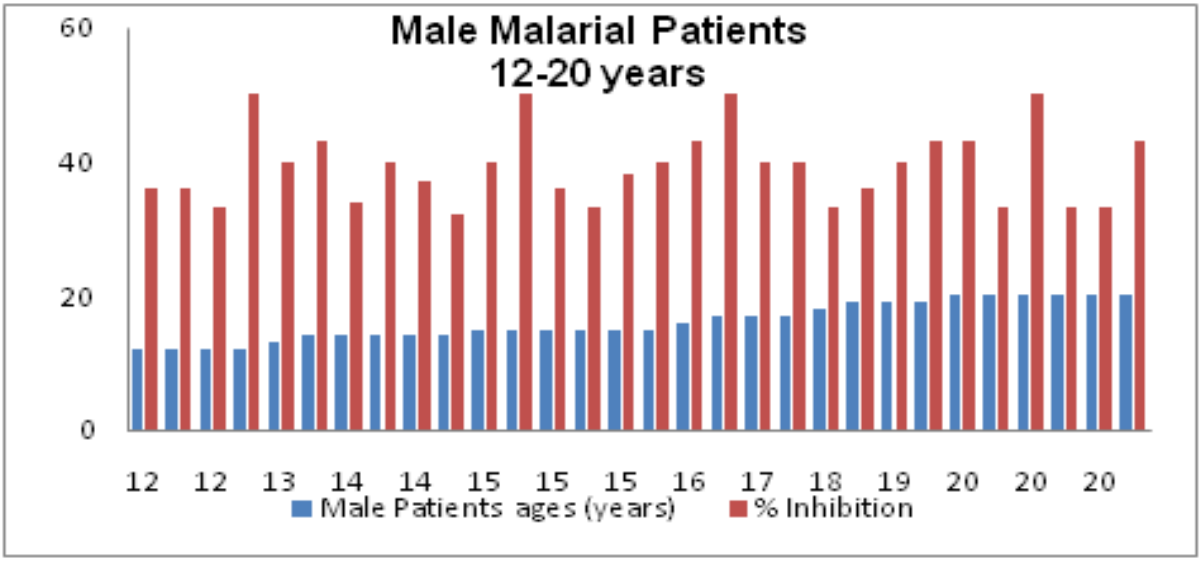

Fig. 1b. Antimalarial effect of chloroform crude extract in male patients (12-20 years).

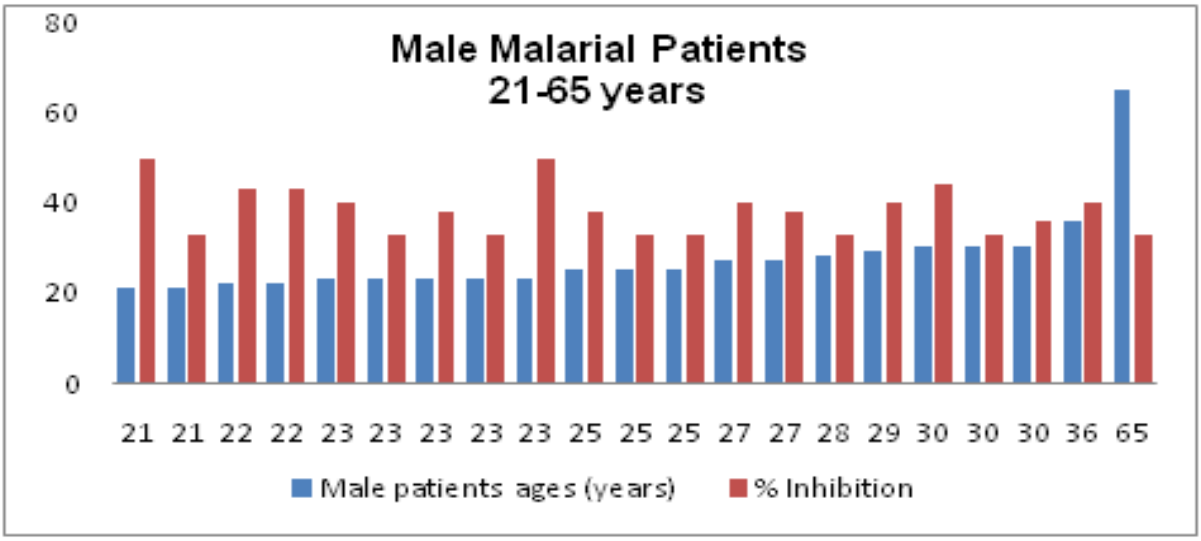

Fig. 1c. Antimalarial effect of chloroform crude extract in male patients (21-65 years).

The distilled water extract applied as a negative (-ive) control showed no effect on malarial parasites, but positive (+ive) control, Nivaquine (synthetic drug) caused cent percent inhibition. In the present study, considerable inhibition of malarial parasites by*Callistemon lanceolatus* crudeextracts was found. In comparison of results obtained in the present study with that of other studies, *Callistemon lanceolatus* results are in agreement with 20, 21, 22, 23, 24, and 25 years patients as*C. lanceolatus inhibited* malarial parasites up to 50%.In comparison with some other previous studies (Peters *et al.,* 1975; Trager and Jensen, 1976; Lorke, 1983; Jurg *et al.,* 1991; El-Tahir *et al.,* 1999; Kraft *et al.,* 2003; Krettli, 2009;) regarding antimalarial activities, plants crude extracts of their different parts like *Cassia alata, Andrographis paniculata,Hedyotis corymbosa,Bridelia cathartica*, *Annona muricata*, *Myrtus communis, Vernonia amygdalina*were usedwhere *Cassia alata, Annona muricata*and *Myrtus communis*inhibited 100,85.61 and 84.8 % at the different concentration of the plants crude extracts.

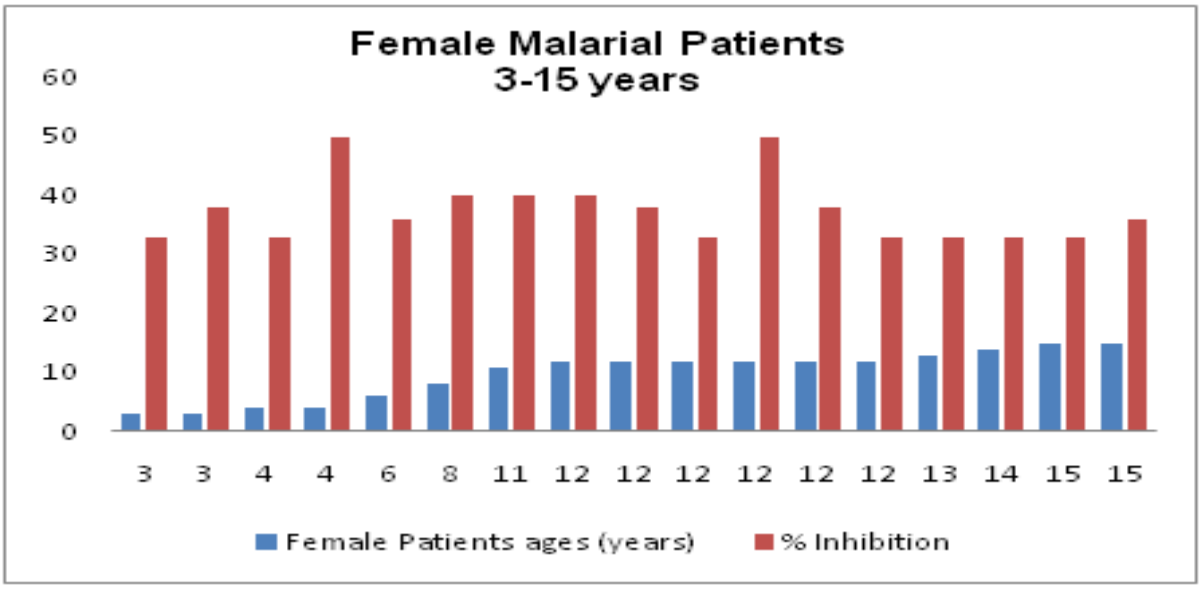

Fig. 2a. Antimalarial effect of chloroform crude extract in female patients (3-15 years).

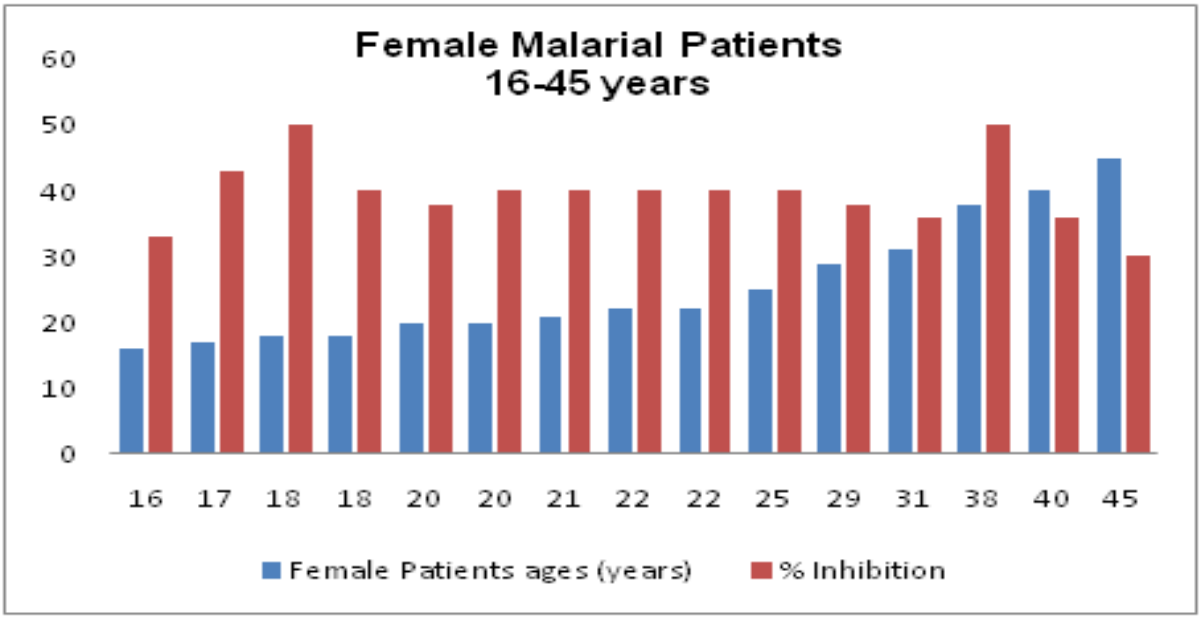

Fig. 2b. Antimalarial effect of chloroform crude extract in female patients (16-45years).

The maximum results showed by *Cassia alata*(100%) onconcentration 128µg/ml while the second *Annona muricata* showed85.61%inhibition on concentration 1000mg/kg. If we increase the quantity of*C. lanceolatus* crude extracts, the resultscan be/will be better thanother plants (Table 3).To the best of our knowledge, there is no antimalarial activity of *Callistemon lanceolatus* but it has shown promising antimicrobial and other biological activities. In many countries especially in India, the plant has been usedby tribalcommunities for the treatment of gastrointestinal disorders, pain, and infectious diseases (Sudhakar *et al.,* 2004).

The methanolic crude extract of *Callistemon lanceolatus* leaves has shown broad spectrum antimicrobial activity against Gram positive bacteria, Gram negative bacteria and some fungal cultures. Antimicrobial activity was measured as zone of inhibition. Crude extract exhibited maximum antimicrobial activity against *Staphylococcus aureus*, followed by *Salmonella typhi, Micrococcus luteus, Candida tropicalis, Pseudomonas aeruginosa* and *Candida albicans. Klebsiella pneumoniae* and *Escherichia coli* were found to be resistant towards the crude methanolic leaf extract of the plant (Rao *et al.,* 2012).*Callistemon lanceolatus* have been comprehensivelyinvestigated scientifically and reported to possess anticholinestrase activity (Gupta, 1997), wound healing activity (Kumar *et al.,* 2007), hepatoprotective activity (Jain *et al.,* 2007), inhibit elastase activity (Kim *et al.,* 2009),cardioprotective activity (Firoz *et al.,* 2011), antiinflammatory activity (Kumar *et al.,* 2011a), antidiabetic activity, hypolipidemic activity and antioxidant activities (Kumar *et al.,* 2011b).

These reports denote the potential of *Callistemon lanceolatus*to be anexcellentfoundation of bioactive compounds with numerous medicinal possessions.

Conclusion

Our findings suggest that chloroform fraction is more useful and potent antimalarial agents can be isolated from itwhich also has antimalarial effects. Still, there is need to test other species of genus *Callistemon* to investigate more potent crude extracts. The more extended study is required to scrutinize the District Bannu to collect adequate data which would help in obtaining an exact picture of the problem.

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