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# The Antimalarial and Antimicrobial Activity and Brine Shrimp Toxicity of *Clematis Brachiata* Extracts

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**The *in vitro* antimalarial activity of the root extract in partly supports the ethnobotanical use of the plant to manage malaria. *Clematis brachiata* Thunberg (Ranunculaceae) is used in Kenya for the management of headaches, malaria and other febrile illnesses, abdominal disorders, yaws and for skin disorders. Old stems and leaves are chewed for the management of toothaches and sore throats. Extracts of the plant were subjected to tests for antimalarial, antibacterial and antifungal activity. The toxicity of the extracts was assessed using the brine shrimp lethality bioassay. The root extract gave the highest *in vitro* antimalarial activity against the mulitidrug resistant strain, *Plasmodium falciparum* VI/S ($IC_{50}$=39.24 μg/ml). The stem and leaf extracts had insignificant antiplasmodial activity. The leaf, stem and root extracts had no bacterial or fungal inhibitory effects even at very high concentrations of 10 mg/ml. The $LD_{50}$ values of the stem and leaf methanol extracts against the brine shrimp larvae was 365.60 and 66.5 μg/ml, respectively.**

**Key Words:** *Clematis brachiata,* Ranunculaceae, antimalarial, antibacterial, antifungal, brine shrimp.

## INTRODUCTION

*Clematis brachiata* Thunberg (Ranunculaceae) is a shrubby climber growing between 700 and 3000 feet above sea level [1]. In Kenya, an infusion of the roots is used to manage diarrhea and causes purgation. The leaves are applied locally to manage unspecified skin disorders and yaws. The fresh flowers and roots are inhaled for the management of headache [2]. In Tanzania, an infusion of the leaves is used to manage malaria while the roots are reported to be highly toxic [3]. An infusion of the fresh juice of the leaves is taken to manage headaches and abdominal disorders [3,4].

The leaves and old stems of *C. brachiata* are chewed by the Kikuyu of Kenya for the management of toothache and sore throat [5]. The leaf macerates and leaf juice from Rwanda showed antimalarial activity [6]. An ethanol-water (80 %) extract of the leaf had potent anti-inflammatory activity [7]. Ethanol extracts of the dried root from Rwanda showed uncertain activity against coxsackie, measles and polioviruses. The extract was inactive against herpes and semlicki-forest viruses [8]. A methanol extract of the leaf (1.0 mg/ml) showed 100 % activity against *Trichomonas vaginalis* [9]. The methanol extract of the dried root (10 mg/ml) from Tanzania was inactive against *Shigella boydii* and *Neisseria gonorrheae* but showed weak activity against *Staphylococcus aureus* [10].

Phytochemical screening shows the presence of tannins, sterols, coumarins, saponins and flavonoids [4]. Available literature shows that no compounds have been isolated from *C. brachiata* to date.

The ethnomedicinal use of the plant to manage malaria, yaws and sore throats prompted the search for antimicrobial activity.

## MATERIALS AND METHODS

### Collection and identification of the plant

The plant was collected in the months of August and September 1997 from Kiambu district, Kenya.

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The plant was authenticated at the National Herbarium, (National Museums of Kenya) and voucher specimens were deposited at the Department of Pharmacology and Pharmacognosy, Faculty of Pharmacy, University of Nairobi. The leaves and stem were dried separately at room temperature and ground to a fine powder.

### Preparation of extracts

Two hundred and fifty grams of the dried leaf, root and stem powder were separately subjected to extraction by cold maceration with 1L of 80-v/v % methanol - water for seven days. The solvent was changed daily. The extract was filtered and reduced to dryness under vacuum. The extracts were used for pharmacological investigations. The yields of the cold methanol extracts of the stem, leaf and roots were 20.0, 17.0 and 40.4 % w/w, respectively, of the dried plant part.

In addition, about 500 g of stem powder was successively subjected to Soxhlet extraction using distilled petroleum ether and methanol each for 48 hours in that order. The extracts were evaporated to dryness under vacuum. The methanol extract was dissolved in about 500 ml of water and partitioned successively with about 2 of petroleum ether (40°C – 60°C), chloroform and ethyl acetate. The three fractions were reduced to dryness under vacuum. The yields of the petroleum ether and methanol extracts were 0.8 % w/w and 14.9 % w/w of the dried stem powder, respectively. The yields of the petroleum ether chloroform and ethyl acetate fractions of the methanol extract were 0.7, 0.3 and 0.6 % w/w of the dried stem powder, respectively.

### Test for antimalarial activity

A chloroquine resistant strain of *Plasmodium falciparum* VI/S was kindly provided by Kenya Medical Research Institute (KEMRI) Parasitology Laboratory. It was cultured in red blood cells suspended in RPMI 1640 (Life Technologies, Grand Island, New York, U. S. A.) solution supplemented with heat-treated frozen-pooled human serum. The *in vitro* antiplasmodial test by inhibition of uptake of radioactive $^3$H-hypoxanthine was carried out as previously described [11]. The $IC_{50}$ was determined using the Q-PRO program provided by Welcome Trust Laboratory, Kenya.

### Brine Shrimp Lethality Bioassay

Brine shrimp (Artemia salina) eggs were obtained from Interpret Ltd., Dorking, England. Marine salt was obtained from Nairobi Pet Shop, Nairobi and baker's yeast from Excel Chemical Ltd., Nairobi. The method used is previously described [12]. The $LD_{50}$ (95% confidence limit) was determined using the Finney Probit Computer Program for analysis of quantal data obtained from Prof. J. C McLaughlin, Purdue University, U.S.A.

### Test for antibacterial and antifungal activity

*Aspergillus niger, Candida albicans , Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the stock cultures of the Drug Analysis and Research Unit (D.A.R.U., Faculty of Pharmacy, University of Nairobi). *Neisseria gonorrheae* was obtained from the Department of Medical Microbiology, University of Nairobi. All bacteria except *N. gonorrheae* were grown on Mueller Hinton medium (Oxoid, Unipath Ltd., Hampshire, England). *N. gonorrheae* was grown on Thayer Martin medium (Becton Dickinson and Co., Cockeysville, MD, USA) without inhibitor under anaerobic conditions. All fungi were grown on Sabouraud Dextrose medium (Topley House, Bury, England). Clotrimazole and ciprofloxacin were provided by D. A. R. U. and were used as the positive controls. Chloroquine diphosphate was obtained from Laboratory and Allied, Nairobi. Sterile water was used as the negative control. Antimicrobial activity was tested by the well diffusion method [13,14].

## RESULTS AND DISCUSSION

### Test for antimalarial activity

The $IC_{50}$ of the cold methanol extract of *Plasmodium falciparum* the root extract against the drug resistant strain is about 39.24 µg/ml. Based on a scale used by Weenen *et al.* [15], the root extract has good antimalarial activity. The leaf extracts did not show any antimalarial

activity. These results support the ethnobotanical use of the roots in Kenya for the management of malaria.

Unlike Kenya, in Rwanda and Tanzania the leaves of *C. brachiata* are used for the management of malaria and the leaf extracts of the plant collected in Rwanda have *in vitro* antimalarial activity [6]. This discrepancy can be explained by the different environmental characteristics such as climate and soil quality leading to different phytochemical characteristics and hence differences in the pharmacological profiles [16].

### Brine shrimp lethality assay

The $LD_{50}$ of various extracts against brine shrimp larvae are presented in Table 1.

**Table 1: The $LD_{50}$ of various extracts of *(clematis brachiata)* against brine shrimp larvae**

| Plant Part | Extraction Method | Solvent | LD50 (µg/ml) |
|---|---|---|---|
| Leaf | Soxhlet | Methanol | 66.50 |
| Stem | Soxhlet | Petroleum ether | 571.70 |
| Stem | Soxhlet | Methanol | 365.60 |
| Stem | Liquid/Liquid Partition | Petroleum ether | 116.60 |
| Stem | Liquid/Liquid Partition | Chloroform | 59.80 |
| Stem | Liquid/Liquid Partition | Ethyl acetate | 23.10 |

The activity of the Soxhlet methanol extract of the leaves ($LD_{50}$ = 66.50 µg/ml) was about seven times that of the stem. The leaves have high activity against brine shrimps. The crude extracts have relatively high lethality and this supports the observation in Tanzania that the plant is generally toxic [3,4]. Out of all the extracts, the ethyl acetate extract of stem had greatest activity against brine shrimps ($LD_{50}$ = 23.10 µg/ml). Spot tests showed that it has the highest amounts of coumarins, flavonoids and anthraquinones. The chloroform extract of the stem had the second highest activity against brine shrimps. Its composition was similar to that of the ethyl acetate extract. The petroleum ether extract of the stem had the least activity against the brine shrimps.

The brine shrimp lethality assay is used to detect compounds with potential cytotoxic and insecticidal activity [17,18,19]. The plant may be a potential source of cytotoxic and insecticidal compounds.

### Antimicrobial activity

None of the extracts of the leaf, stem and roots showed any antibacterial or antifungal activity. Lack of antimicrobial activity was unexpected since the plant is used in the management of skin disorders, sore throats and yaws. A previous study showed that methanol extracts of the dried root (10 mg/ml) of the plant collected in Tanzania was inactive against *Shigella boydii* and *Neisseria gonorrheae* but showed weak activity against *Staphylococcus aureus* [10].

## CONCLUSION

The root extract of *C. brachiata* has good *in vitro* antimalarial activity. This in part supports the ethnobotanical use of the roots in Kenya for the management of malaria. The leaf extract showed very high activity against brine shrimps and could be a potential source of bioactive compounds. The plant lacks antibacterial and antifungal activity.

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## REFERENCES

[1] H. Beentje, *Kenya Trees and Shrubs and Lianas*. National Museums of Kenya, Nairobi, 1994, p 59.

[2] J.O. Kokwaro, *Medicinal Plants of East Africa*. Kenya Literature Bureau, Nairobi, 1993, p131.

[3] H.P. Neuwinger, *African Ethnobotany, Poisons and Drugs*. Chapman and Hill. Weinheim, 1994, p 754-756.

[4] S.C. Chhabra, R.L.A. Mahunnah and E. N. Mshiu, *Ethnopharmacol.* 33 (1984) 143-157.

[5] F.N. Gachathi, *Kikuyu Botanical Dictionary*. GTZ, Nairobi, 1989, p 64.

[6] E. Hakizamungu and L. van Puyvelde, *Bull De Liason ACCT: Médecine Traditionelle et Pharmacopée*. 2 (1988)11-17.

[7] A. Lasure, B. van Pole, L.S. Clerck, C.H. Bridts, W.J. Stevens, P.C. Rwangaro, I. Pieters and A.J. Vlietinck, *Phytomedicine* 1 (1995)303-307.

[8] A.J. Vlietinck, L. van Hoof, J. Totte, A. Lasure, D. Vanden Berghe, P.C. Rwangaro and J. Mvukiyumawani, *Ethnopharmacol.* 46 (1995) 31-47.

[9] E. Hakizamungu, van Puyelde and M. Wery, *Ethnopharmacol.* 36 (1992) 143-146.

[10] S.C. Chhabra and F.C. Usio, *Fitoterapia* 626 (1991) 499-503.

[11] H.A. Oketch-Rabah, E. Lemmich, S.F. Dossaji, G.T. Theander, C.E. Olsen, C. Cornet, A. Kharamzmi and S.B. Christensen, *J. Nat. Prod.* 60 (1997) 458.

[12] J.W. Mwangi, W. Masengo, G.N. Thoithi, I. O. Kibwage, *East and Cent. Afri. J. Pharm. Sci.* 2 (1999) 63-71.

[13] R. Cruickshank, *Medical Microbiology: Guide to Diagnosis and Control of Infection*, 11th edition, E. and S. Livingston Ltd.,Edinburgh and London, 1968, p 888.

[14] A.W. Bauer, W.M.M. Kirby, J.C. Sherries and M. Truck, *Am. J. Clin. Pathol.* 45 (1966) 493.

[15] H. Weenen, M.H.H. Ngunya, D.H. Bray, L.B. Mwasumbi, L.S. Kinabo, V.A.E. and B. Kilimali, *Planta Med.* 56 (1990) 368-370.

[16] R. Capasso, A.A. Izzo, L. Pinto, T. Bifulco, C. Vitobello and N. Mascolo, *Fitoterapia,* 71 (2000) S58-S65.

[17] J.L. McLaughlin, C.J. Chang and D.L. Smith, *Studies in Natural Products Chemistry*, Vol. 9. Atta-ur-Rahman (Ed.) Elsevier Science Publisher B. V., Amsterdam. 1991.

[18] J.L. McLaughlin J.L. *Proceedings, NIH Workshop, Bioassays for Discovery of Antitumour and Antiviral Agents from Natural Sources, Bethesda*, Oct. 18 –19, 1988, p 22.

[19] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin *Planta Medica,* 45 (1982) 31 - 34.