

## **Preliminary Phytochemical Screening and Antimalarial Studies of *Spathodea campanulatum* P. Beauv Leaf Extracts**

**Rangasamy Dhanabalan<sup>1\*</sup>, Asirvatham Doss<sup>1</sup>, Muthusamy Jagadeeswari<sup>1</sup>, Rajamanickam Karthic<sup>1</sup>,  
Muthusamy Palaniswamy<sup>2</sup> and Jeyaraman Angayarkanni<sup>2</sup>**

Department of Microbiology<sup>1</sup>, RVS College of Arts and Science, Coimbatore, Tamilnadu, India

Department of Microbiology<sup>2</sup>, Karpagam Deemed University, Coimbatore, Tamilnadu, India

Department of Microbial Technology<sup>3</sup>, Bharathiyar University, Coimbatore, Tamilnadu, India

\*Corresponding author: bharathi.dhanabalan@gmail.com

Issued 30 October 2008

### **ABSTRACT**

*Spathodea campanulata* P. Beauv is extensively used in Indian traditional and folklore medicines to cure various human ailments. The preliminary phytochemical screening of the leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids and terpenoids. The organic leaf extracts of this species was evaluated for antimalarial activity against clinically isolated *Plasmodium falciparum* chloroquine-resistant and sensitive strains. The *in vitro* antimalarial activity was performed following schizont maturation inhibition assay, (SMI). The ethanol fraction exhibited very good antiplasmodial activity against both chloroquine sensitive and resistant *Plasmodium falciparum* isolates followed by the butanol and chloroform extracts.

**Key words:** *Spathodea campanulata*, Antimalarial activity, Schizont maturation assay and ethanol fraction.

### **INTRODUCTION**

Malaria is the worldwide most important parasitic disease with an incidence of almost 300 millions clinical cases and over one million deaths per year. There were an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly of children under 5 years. 109 countries were endemic for malaria in 2008, 45 within the WHO African region (WHO, 2008). While we wait for malaria vaccine, effective chemotherapy remains the mainstay of malaria control (Winstanley, 2000). *Plasmodium falciparum*, the potentially lethal malaria parasite has shown itself capable of developing resistance to nearly all used antimalarial drugs and resistant strains have rapid extension (Plowe *et al.*, 1995). The lost of effectiveness of chemotherapy constitute the greatest threat to the control of malaria. Therefore, to overcome malaria, new knowledge, products and tools are urgently needed; especially new drugs are required (Omulokoli *et al.*, 1997; Rasoanaivo *et al.*, 1999).

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies

(UNESCO, 1998). The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand.

*Spathodea campanulata* is a species belonging to the Bignoniaceae family, native from equatorial Africa. The Siddha/Tamil name of this species is Patadi and in folk it is popularly called as Ruugatuuraa. It is very commonly found and planted in the coffee estates of Munnar, South Tamilnadu and denoted by the name Malaria Maram(tree). In English the species is called as Syringe tree, Fountain tree, African Tulip tree. It is a medium-size tree (15-25 m high), characterized by red garish flowers. It is often employed in gardening in tropical and subtropical areas, including South America (Joly, 1985). Flowers and stem bark extracts have shown molluscicidal activity and also employed in diuretic, anti-inflammatory treatments. The leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches and diarrhea (Jardim *et al.*, 2003; Mendes *et al.*, 1986). Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts (Niyonzima *et al.*, 1999; Makinde *et al.*, 1988).

Several phytochemical studies were performed with different parts of *S. campanulata*, including stem barks, leaves, flowers and fruits (Niyonzima *et al.*, 1999; Ngouela *et al.*, 1990; Ngouela *et al.*, 1988; Amusan *et al.*, 1995; Amusan *et al.*, 1996). The leaves have furnished spathodol, caffeic acid, other phenolic acids and flavonoids (Ngouela *et al.*, 1991; Subramanian *et al.*, 1973; El-Hela, 2001a; El-Hela, 2001b). Banerjee and De (2001) showed the presence of anthocyanins in flowers of *S. campanulata*. A qualitative fungitoxic activity of *S. campanulata* roots against *Cladosporium herbarum* CCT 0279 has been evaluated and reported (Pianaro *et al.*, 2007). *In vitro* antibacterial activity of leaf extracts of this plant was evaluated (Parek.J and Chanda.S, 2007). No reports on antimalarial activity of *S. campanulata* leaf extracts/fractions have been reported till date. Thus, the present work is aimed to evaluate the antimalarial activity of *S. campanulata* leaf extracts/fractions against the clinically isolated *Plasmodium falciparum* chloroquine-resistant and sensitive strains.

## **MATERIAL AND METHODS**

### **Plant materials**

Fresh plant leaf samples were collected from the Munnar Kundala Tea Estates, Kerala, India during March 2008. The taxonomic identity of the plant was confirmed by Botanical Survey of India, Southern Circle, Coimbatore, India and the voucher specimen of the plants was preserved in RVS College Microbiology Laboratory. Fresh plant material was washed under running tap water, air dried, homogenized to fine powder and stored in airtight bottles.

### **Preparation of crude extracts and fractions**

Crude extraction with 50% ethanol was done using soxhlet apparatus and cold crude extract was prepared with 80 % ethanol (Klayman, 1985). For solvent extraction, 50 g of air-dried powder was taken in 250 ml of organic solvent (Chloroform, butanol, hexane, ethanol and ethyl acetate) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated at room temperature. The procedure was repeatedly followed for all the solvents individually. The extracts were stored at 4<sup>0</sup>C in airtight bottles.

### **Phytochemical Screening Test**

The phytochemical screening of the plant extract was carried out by following methods used by Amarasingham *et al.*, (1964), Das and Bhattacharjee, (1970), Gibbs, (1974), Trease and Evans (1978), Santaram and Harborne (1984) to detect the presence or absence of certain bioactive compounds.

### **Antiplasmodial activity**

#### **Preparation of medium for the cultivation of malaria parasite**

Around 10.4 g of RPMI 1640 containing 25mM of triple HEPES buffer was dissolved in 960 ml of triple distilled water. To this 2g of glucose and 40µg/ml of gentamycin sulphate were added. The solution was sterilized by passing through a Millipore filter of 0.22µm porosity and stored at 4°C as 96 ml aliquots in glass media bottle. An incomplete media (washing media) was prepared by adding 4.2 ml of freshly prepared 5% sodium bicarbonate to 96 ml of stock RPMI 1640. Simultaneously, AB<sup>+</sup>ve blood serum without anticoagulant was aseptically collected placed in aliquots. The serum was inactivated in 56°C water bath/half an hour and stored in deep freezer at -20 °C. A complete media was prepared by adding 10 ml of normal inactivated AB<sup>+</sup>ve human serum to 90 ml of incomplete media. A<sup>+</sup>ve blood was collected with anticoagulant and the RBCs were pelleted by centrifugation to which equal amount of complete media was added and stored for use when required.

#### **Collection of *Plasmodium falciparum* positive blood samples from the field**

The parasite collection was carried out in RVS Hospital Diagnostic Lab and Government Hospital, Palladam. Prior to collection peripheral blood smears of patients with no history of intake of antimalarials was checked for the presence of healthy asexual parasites. 3ml of blood was drawn from the positive patients and centrifuged at 1500 rpm/10 in a heparinised centrifuge tube. Supernatant/plasma was removed and the cells were suspended in an equal volume of cryoprotectant (28ml of glycerol to 72 ml of 4.2% sorbitol in normal saline and filter sterilized) and then distributed in cryotubes and frozen quickly in liquid nitrogen (-196 °C). Initiation of culture and sub-culturing was performed whenever required for tests (Connelly *et al.*, 1996).

#### **Revival of Cryopreserved parasites**

The culture vials from liquid nitrogen was thawed quickly in water bath at 37 °C, the contents were centrifuged at 1500 rpm/10 min and equal volume of NaCl (3.5%) was added by removing the supernatant. The suspension was centrifuged again, supernatant removed and the pellets were washed twice with complete medium supplemented with 15% serum. After washing the cryopreserved cells the culture was setup by adding fresh washed erythrocytes.

#### ***In vitro* screening of extract/ fraction against *Plasmodium falciparum***

The cultures was synchronized using 5% aqueous solution of sorbitol (1 portion of pellet and 9 portion of sorbitol) and kept for 5-7min in room temperature to ensure the killing of all other stages of parasite except ring forms. The whole suspension was centrifuged at 1500 rpm/5 min and the pellets were washed with incomplete media twice. The parasitaemia was adjusted to about 1% for the assay by diluting with fresh washed RBC's. The plant material to be tested was dissolved in DMSO so that the concentration of DMSO never exceeded 0.1% in the experiment. The stock solution was diluted with RPMI1640 to obtain different concentrations.

The test was performed in 96 well plates using chloroquine sensitive and resistant isolates isolated from the patients. Different concentration of crude extracts and fractions were dispensed in 96 well plates in duplicate. The first well in all rows were without any drug but with DMSO and considered to be control. The synchronized parasites were inoculated in all the wells to get a final concentration of 5% haematocrit. The plates were incubated at 37°C for 24-30 hours depending on the maturation of the schizont. After conformation of the schizont maturation, smears were prepared from the wells. The experiment is considered to be invalid, if the schizont maturation is below 10% (Draper *et al.*, 1988). The smears were stained in Giemsa stain and numbers of schizonts were counted/200 asexual parasites.

The values were compared between control and test wells. The inhibition percentage of schizont for each concentration of extract/fraction was calculated as follows (Anon, 1979).

Inhibition = 100 - A      A: percentage growth of schizont in test wells which can be determined by

$A = \frac{z}{m} \times 100$       Z: number of schizont/200 asexual parasites in the test wells

M: number of schizonts in the test wells

M: number of schizonts in the test wells

From dose response curve, IC<sub>50</sub> values (concentration at which inhibition of parasite growth represents 50%) were calculated from the plot of probability of chloroquine activity and logarithm of drug concentration by linear regression analysis (Sharma Poonam, 1999).

## RESULTS

### Screening tests of plant extracts/fractions

Crude ethanol extracts (50% and 80%) were prepared from *Spathodea campanulatum* and subjected to fractionation using ethanol, chloroform, butanol, hexane and ethyl acetate. The percentage yield obtained is given in Table 1. Preliminary phytochemical analysis of the leaf extracts of *Spathodea campanulatum* revealed the presence of flavonoids, alkaloids, terpenoids, saponins, tannins, steroids and cardio amino glycoside Table 2. The presence of alkaloids, flavonoids, tannins and steroids, terpenoids were detected in ethanol and hexane extracts respectively. Saponins were detected in water extracts.

### Antiplasmodial activity

The crude ethanol (50% and 80%) extracts and fractions of *Spathodea campanulatum* were tested *in vitro* for its antimalarial activity using clinically isolated laboratory adapted chloroquine sensitive and resistant *Plasmodium falciparum* parasites. The assay followed was schizont maturation inhibition assay, (SMI). The specific changes in morphology produced by particular extract may hint at different modes of action of the putative active principles in the extracts. The parasitaemia also decreased with increasing concentration of the extract reflecting an inhibitory activity on parasitic replication. This may be indicative of a significant potential for isolating purer compound.

The crude extract (both 50% and 80%) of *Spathodea campanulatum* tested *in vitro* did not show profound antimalarial activity. The IC<sub>50</sub> value of crude ethanol extract (50%) against chloroquine sensitive *Plasmodium falciparum* isolates was 88.3±2.83µg/ml where as the IC<sub>50</sub> value against chloroquine resistant *Plasmodium falciparum* isolates was 108.2±8.20µg/ml. The IC<sub>50</sub> value of crude ethanol extract (80%) against chloroquine sensitive *Plasmodium falciparum* isolates was 68.5±10.60µg/ml and against chloroquine resistant *Plasmodium falciparum* isolates was 100.8±3.53µg/ml. The chloroform, butanol, hexane, ethanol and ethyl acetate fractions when tested *in vitro* showed IC<sub>50</sub> value of 18.7±2.23µg/ml, 12.3±1.32µg/ml, 90.0±11.12µg/ml, 7.5±1.25µg/ml and 14.2±1.20µg/ml respectively against chloroquine sensitive *Plasmodium falciparum* isolates and the IC<sub>50</sub> value for chloroquine resistant *Plasmodium falciparum* isolates was 19.0±2.42µg/ml, 21.3±1.82µg/ml, 118.3±2.23µg/ml, 8.9±1.30µg/ml and 51.0±3.10µg/ml respectively (Table 3)

## DISCUSSION AND CONCLUSION

Crude extracts are simplest of available of medications and are still promoted by WHO policies as emerging alternative systems of medicine to reach the large population not covered by formal medical care in remote areas. In this regard the result of this preliminary study is very much encouraging. This is the first report on *in vitro* antimalarial effect of *Spathodea campanulatum* using leaf extracts (Table 3). Previous reports on this plant leaf extracts have specified only about *in vitro* antibacterial activity (Parek.J and Chanda.S, 2007). Stem bark extracts were used for purgative, antiviral and antimalarial works (Niyonzima *et al.*, 1999) and roots in fungitoxic activity (Pianaro *et al.*, 2007). In the present work crude plant extracts did not show profound antimalarial activity, upon fractionation has yielded purer compounds with potent antimalarial activity (Wright and Phillipon, 1990). The ethanol fraction exhibited very good antiplasmodial activity against both chloroquine sensitive and resistant *Plasmodium falciparum* isolates followed by the butanol and chloroform extracts. Further studies can be carried out for the isolation of active principle and elucidation of chemical structure with an objective of exploring the possibility of using the component as oral/parenteral drug for treating malarial infection.

## REFERENCES

- Amarasingam, R.P., Bisset, N.G., Millard, A.K. and Woods, M.C, (1964). Phytochemical survey of Malaya, part III. Alkaloids and Saponins, *J. Ecorn.Bot.* 18:270-278.
- AMUSAN, O. O. G.; ADESOGAN, E. K.; MAKINDE, J. M. Antimalarial active principles of *Spathodea campanulata* stem bark. *Phytotherapy Research*, London,
- AMUSAN, O. O. G.; MSONTHI, J. D.; MAKHUBU, L. P. Molluscicidal activity of *Spathodea campanulata*, *Andrachne ovalis*, *Phytolacca dodecandra* and *Hypoxis rooperi*. *Fitoterapia*, Amsterdam, v.66, p.113-116, 1995.
- Anon, 1979. Quinghaosu Antimalarial coordinating Research group- Antimalarial studies on Quinghaosu. *Chin. Med. J.* 92: 811-816.
- BANERJEE, A.; DE, B. Anthocyanins in some flowers of West Bengal. *Journal of Medicinal and Aromatic Plant Science*, Lucknow, v.23, p.600-604, 2001.
- Connelly, M.P., Fabiano, E., Patel, I.H., Kinyanjui, S.M., Mberu, E.K., Watkins, W.M., 1996. Antimalarial activity in crude extracts of Malawian medicinal plants. *Annals of Tropical Medicine and Parasitology* **90**:597-602
- Das, A.K. and Bhattacharjee, A.K, (1970). A systematic approach to Phytochemical Screening, *Trop. Sci.*1254-

- Draper C C, Brubaker G, Geser A, Kilimali V A, Wernsdorfer W H. Serial studies on the evolution of chloroquine resistance in an area of East Africa receiving intermittent malaria chemosuppression. *Bull W H O.* 1985; **63**: 109–118.
- Draper, C.C., Hills, M., Kilmali, A.E.B. and Brubaker, G.1988. Serial studies on the evolution of drug resistance in malaria in an area of east Africa: Findings from 1979 upto 1986. *J. Trop. Med. Hyg.*, 91: 265-273.
- El-Hela, A. A. A new iridoid glucoside from *Spathodea campanulata* Beauv. leaves. *Al-Azhar Journal of Pharmaceutical Sciences*, Cairo, v.27, p.115-120, 2001b.
- El-Hela, A. A. Phenolics from *Spathodea campanulata* Beauv. Leaves. *Al-Azhar Journal of Pharmaceutical Sciences*, Cairo, v.27, p.152-162, 2001a.
- Gibbs, R.D, (1974), *Chemotaxonomy of flowering plants*. Vol. 1McGill-Queens University Press- N, Montreal-London.
- Jardim, M. A.; Mendonça, L. F. R.; Ferreira, M.M. D. *Os produtos naturais para o desenvolvimento sustentável biotecnológico*. Disponível em: <http://www.mdic.gov.br/tecnologia/revistas/artigos/200112pa/mariojardim.pdf>.>. Acesso em: 20 dez. 2003.
- Joly, A. B. *Introdução à taxonomia vegetal*. 7. ed. São Paulo: Ed. Nacional, 1985. 503p.
- Klayman, D.L. 1985.Quinghaosu (Artemisinin): An antimalarial drug from china. *Science*, 228: 1049-1054.
- Makinde, J. M.; Amusan, O. O. G.; Adesogan, E. K. The antimalarial activity of *Spathodea campanulata* stems bark extract on *Plasmodium berghei berghei* in mice. *Planta Medica*, Stuttgart, v.54, n.2, p.122-125, apr. 1988.
- Mendes, N. M.; Souza, C. P.; Araújo, N.; Pereira, J. P.; Katz, N. Atividade moluscicida de alguns produtos naturais sobre *Biomphalaria glabrata*. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.81, p.87-91, 1986.
- Ngouela, S.; Nyasse, B.; Tsamo, E.; Sondengam, B. L.; Connolly, J. D. Spathodic acid: a triterpene acid from the stem bark of *Spathodea campanulata*. *Phytochemistry*, Oxford, v.29, n.12, p.3959-3961, 1990.
- Ngouela, S.; Tsamo, E.; Sondengam, B. L. Extractives from Bignoniaceae: constituents of the stem bark of *Spathodea campanulata*. *Planta Medica*, Stuttgart, v.54, n.5, p.476, oct. 1988.
- Ngouela, S.; Tsamo, E.; Sondengam, B. L.; Connolly, J. D. Spathodol, a new polyhydroxysterol from the leaves of *Spathodea campanulata*. *Journal of Natural Products*, Washington, v.54, n.3, p.873-876, may 1991.
- Niyonzima, G.; Laekeman, G.; Witvrouw, M.; Van Poel, B.; Pieters, L.; Paper, D.; Clercq, E.; Franz, G.; Vlietinck, A. J. Hypoglycemic, anticomplement and anti-HIV activities of *Spathodea campanulata* stem bark. *Phytomedicine*, Jena, v.6, n.1, p.45-49, mar. 1999.
- Omulokoli, E., Khan, B. and Chhabra, S. C. (1997). Antiplasmodial activity of four Kenyan medicinal plants. *J Ethnopharmacol.* **56**:133–137
- Parekh J and Chanda S. (2007). *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *African Journal of Microbiology Research* Vol. 1 (6) pp.092-099.
- Phytochemical, UNESCO (1996). *Culture and Health*, Orientation Texts –World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France, pgs. 129.
- Pianaro, A; Dalva Trevisan Ferreira; Jurandir Pereira Pinto; Noemia Kazue Ishikawa; Raimundo Braz-Filho. Iridoid glucoside and antifungal phenolic compounds from *Spathodea campanulata* roots. *Semina: Ciências Agrárias*, Londrina, v. 28, n. 2, p. 251-256, abr./jun. 2007

- Plowe, C. V., Djimde, A., Bouare, M., Doumbo, O. and Wellens, T. E., (1995). Pyrimethamine and proguanil resistance-conferring mutation in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *Am. J. Trop. Med. Hyg.* **52 (6)**: 565-568
- Rasoanaivo, P., Ratsimamanga-Urverg, S., Ramanitrhasimbola, D., Rafatro, H. and Rakoto-Ratsimamanga A., (1992). Criblage d'extraits de plantes de Madagascar pour recherche d'activité antipaludique et d'effet potentialisateur de la chloroquine. *J Ethnopharmacol* **64**:117–126.
- Santaram and Harborne, J.B, (1984) (Eds.) *Phytochemical method* a Chaman and Hall, London.
- Sharma Poonam. 1999. In vitro antimalarial and antiamebic potential of plants of medicinal value present in desert ecosystem- A case study from Rajasthan. Ph.d. Thesis, Jahwaharlal Nehru University- New Delhi-67, India.
- Subramanian, S. S.; Sulochana, N.; Nagarajan, S. Caffeic acid from the leaves of *Spathodea campanulata*. *Current Science*, Bangalore, v.42, n.11, p.403, 1973.
- Trease. G.S., and Evans, H.C, (1978). *Textbook of Pharmacognosy*. 9<sup>th</sup> edition. Bailiar Zindall And Co., London.
- UNESCO (1998). FIT/504-RAF-48 Terminal Report:*Promotion of Ethnobotany and the Sustainable Use of PlantResources in Africa*, pgs. 60, Paris, 1998.v.10, n.8, p.692-693, 1996.
- WHO Library Cataloguing-in-PublicationData World malaria report 2008.“WHO/HTM/GMP/2008.1”.
- Winstanley, P. A., (2000). Chemotherapy for falciparum malaria: the armoury, the problems and the prospects. *Parasitol. Today* **16**: 146-153.
- Wright, C.W. and Phillipson, J.D.1990. Natural product and the development of selective antiprotozoal drugs. *Phytother. Res.*; 127-139.

**Table 1. Preparation of crude extracts from *Spathodea campanulatum*.**

Fractions	Method of extraction	% Yield
Crude extract 50% ethanol	Soxhlet apparatus	42.18
Crude extract 80% ethanol	Cold extraction	32.28

**Table 2. The phytochemical profile of the leaf extracts *Spathodea campanulatum*.**

Phytochemicals	Presence/Absence
Sugar	+
Protein	+
Alkaloids	+
Flavonoids	+

Saponins	+
Tannins	+
Steroids	+
Cardiac Glycoside	-
Terpenoids	+
Lipids	-

**Table 3. *In vitro* antiplasmodial effect of the crude extract/fractions of *Spathodea campanulatum* against chloroquine resistant and sensitive *Plasmodium falciparum* isolates.**

<i>Spathodea campanulatum</i> Extract/fractions	IC <sub>50</sub> value	
	Sensitivity	Resistant
Crude extract(50% ) ethanol	88.3±2.83	108.2±8.20
Crude extract(80% ethanol	68.5±10.60	100.8±3.53
Chloroform fraction	18.7±2.23	19.0±2.42
Butanol fraction	12.3±1.32	21.3±1.82
Hexane fraction	90.0±11.12	118.3±2.23
Ethanol fraction	7.5±1.25	8.9±1.30
Ethyl acetate fraction	14.2±1.20	51.0±3.10µg