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# PHYTOCHEMICAL PROFILING AND LARVICIDAL POTENTIAL OF CRUDE ROOT EXTRACTS OF *CENTELLA ASIATICA* L. (THANKUNI), AGAINST THE MOSQUITO *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE)

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## ABSTRACT

Phytochemical profiling of the crude extract from the roots of *Centella asiatica* L. was carried out according to HPTLC analysis method. Phytochemicals found in the extracts were identified as Asiatic acid. These crude root extracts of Thakuni, *Centella asiatica* L., was further investigated for its anti-mosquito potential as larvicidal *Aedes aegypti*, the vector of dengue haemorrhagic fever. The ethanol-extracted phytochemicals from the roots of *Centella asiatica* possessed larvicidal activity against fourth instar larvae of *Ae. aegypti* with $LD_{50}$ and $LD_{95}$ values of 140.0 and 200.0 mg/L, respectively. The abnormal movement observed in treated larvae indicated that the toxic effect of *Centella asiatica* extract was probably on the nervous system. *Centella asiatica* root extracts, thus was found with potential larvicidal activity against the fourthinstar larvae of *Ae. Aegypti* and further can be considered as a probable source of some biologically active compounds used in the development of mosquito control agents, particularly larvicidal products.

## KEYWORDS

*Centella asiatica*, *Aedes aegypti*, Phytochemistry, Larvicidal, Asiatic acid, Asiaticoside, and Madecasoside.

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## INTRODUCTON

*Centella asiatica*, or locally known as thakuni, is an aromatic plant of the family Apiaceae. In folk medicine it is used to treat a wide range of illness[1]. Fresh leaves are chewed in case of tonsillitis, throat trouble, pneumonia. The entire plant is used for memory improvement and stomach disorder. Extraction from the leaves can also be mixed with coconut oil and applied on the head before bath and as regular hair oil. This strengthens the hairs follicles and promotes hair growth. The whole plant is eaten after cooking or raw for cough, fever, blood

dysentery, cholera, stomachache, boil, rheumatism, blood purification and as tonic. The leaf extract is also used for skin disease, headache and expelling worms. Tribals use the dry powdered leaves of the plant is good for the tuberculosis and the recommended dose in two tablespoons every 2 hours. The decoction or infusion of the plant is used for leprosy, skin diseases; chronic inflammation of the skin, chronic ulcers, contagious sores, chronic rheumatism and piles. Tablets made from 10 gm of root with 2gm of that of *Dipteracanthus prostratus* (dhamani) taken twice daily (morning and afternoon) to treat dysentery.

One study has even proved that oral administration of asiatic acid influences % parasitaemia suppression, ameliorated malarial anaemia and increased biophysical properties on infected animals[2]. The objective of this study is to investigate the role of asiatic acid which is found in quantities more than asiaticoside and madecasoside in the roots as compared to other aerial parts of the plant. Foreseeing its potential root extracts has been chosen as the main interest in this study, since it contains asiatic acid as the major component found in the study.

*Aedes aegypti* (L.) is a known vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. Dengue fever has become a significant public health problem as the number of reported cases have risen sharply like dengue haemorrhagic fever and dengue shock syndrome, along with certain manifestations of the central nervous system involvement[3,4]. Now a days more than two-fifths of the world's population are now at risk of dengue.

A lot of research on the potential of natural plants to protect against mosquitoes and other insect pests[5] have been done. Since the continued applications of synthetic compounds have serious drawbacks, including the widespread development of insecticide resistance along with disturbances in the biodiversity.

**Dengue in Global Scenario**

Dengue fever is a very old disease. An estimated 2.5 billion people are at risk and 50 million dengue infections occurring annually. The relationship of countries with Dengue has long and intense. Worldwide nearly 2.5-3billion people (40% of the global population) continue to live at constant risk of contracting infection. While 50 million cases and 22,000 deaths are estimated to regularly occur annually in 100 endemic countries worldwide, including hospitalization of nearly 500,000 cases of which 90% are children. The South East Asia region contributes 52% or 1.3 billion cases annually.

**Dengue in Indian scenario**

The first report of existence of dengue fevers in India was way back in 1946[6]. In 1963-1964, an initial epidemic of dengue fever was reported on the Eastern Coast of India, it spread northwards and reached Delhi in 1967[7] and Kanpur in 1968[8]. The first epidemic of clinical dengue like illness was recorded in Madras in 1780. The first confirmed report of Dengue infection in India dates back to 1940s and thereafter several states began to report the disease which mostly struck in epidemic proportion often in inflicting heavy morbidity and mortality both in urban and rural environments. The first DHF outbreak occurred in Calcutta (Kolkata, West Bengal) in 1963 with 30% of cases showing haemorrhagic manifestations. In 2013 up to the month of October 55, 063 cases and 138 deaths due to dengue was reported in India. Of these, 3, 283 cases and 4 deaths were reported from West Bengal[9]. Upto mid November 2016 a total of 22659 cases of dengue / DHF were reported in the state of West Bengal with 45 deaths[10].

## MATERIAL AND METHODS

**Plant extract**

Roots of *Centella asiatica* were obtained from local sources and were authenticated by Dr. Sharmistha Gupta, Junior Scientist, Department of Higher Education Science and Technology and Biotechnology, Government of West Bengal vide voucher no. DHEST and BT/H/SB01-18/WBSCST. Roots were cleaned shade dried and chopped and

powdered to a moderate size. This powered material was successively extracted three times by maceration, with4.5 L of 95% ethanol at room temperature for 2 d each time. Anethanolic extract was suction filtered through a Buchner funnel and the combined filtrates were concentrated by a rotary evaporator at 45°C until the solvent completely evaporated. The residue of ethanol-extracted *Centella asiatica* was thus obtained, lyophilized, and then kept at -20°C until testing for larval toxicity studies.

**Phytochemical Analysis**

The major secondary metabolites like carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, glycosides, saponins, quinons and phenolic compounds were analysed[11]. The study was done with respect to the available standard protocols.

**Test for Alkaloids**

**Wagner's reagent test**

A fraction of the extract was treated with 3 -5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate which indicates the presence of alkaloids.

**Mayer's test**

To about 3 ml. of extract, a few drops of Mayer's reagent are added and observed for the formation of cream coloured precipitate.

**Dragendorff's test**

To about 3 ml of extract, a few drops of Dragendorff's reagent are added and observed for the formation of orange or orange red **precipitate.**

**Hagers Test**

2ml solution of the extract and 0.2ml of dilute Hydrochloride acid were taken in a test tube. Then 1ml of Hager's reagent was added to it and observed for the formation of yellow crystalline precipitate.

**Test for Carbohydrate**

**Molish's Test**

To 2 ml of Molish reagent, 2 ml of the extract were added and shaken well. To this 2 ml. of conc. $H_2SO4$ was added through the sides of the test tube. Appearance of a reddish violet ring at the junction of the two layers indicate the presence of carbohydrates.

**Fehling's Test**

The filtrate was treated with 1 ml. of Fehling's A and B and heated in a boiling water bath for 5 -10 minutes. Appearance of a reddish orange precipitate shows the presence of carbohydrates.

**Detection of Flavanoids**

The extracts were treated with conc. $H_2SO4$ and observed for an yellowish orange colour for the presence.

**Test for cardiac Glycosides**

Add 1ml. of extract with 1 ml. of glacial acetic acid and 2-3 drops of 5% ferric chloride solution. To this mixture were added 0.5 ml. dilute HCl. Appearance of a green ring which first turns to violet and then to brown at the interface indicates the presence of cardiac glycosides.

**Liebermann's Test**

We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added $H_2SO_4$ concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

**Keller-Kiliani Test**

A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 ml aqueous plant extract and 1 ml $H_2SO_4$ concentrated. A brown ring supposed to be formed was not found between the layers which showed the entity of cardiac steroidal glycosides.

**Salkowski's Test**

We added 2 ml $H_2SO_4$ concentrated to the whole aqueous plant crude extract. A reddish brown color formed which indicated the presence of steroidal aglycone part of the glycoside.

**Test for protein**

**Biuret Test**

One ml of 40% Nacl. and 2 drops of 1% Cuso4 were added to the leaf extracts. Appearance of a violet colour confirms the presence of proteins. Test for phenolic Compounds:-

**Ferric chloride Test**

Two ml of diluted extracts were treated with dil. Fecl3 solution. Appearance of a violet colour indicate the presence of phenol like compounds.

**Test for quinines**

A small amount of the extract was treated with conc. Hcl. and observed for the formation of a yellow precipitate colouration.

**Test for Saponins**

Two ml of the extracts were diluted with 20 ml of distilled Water, shaken vigoursly and was observed for a stable persistent froth.

**Test for steroids**

To1ml. of solvent extract in a test tube acetic anhydride was added and kept in a boiling water bath for 5min, then cooled followed by the addition of 1ml of Con. $H_2SO4$ along the sides of the test tube. Appearance of a green color indicate that the occurrence of steroids

**Test for tannins**

To the extracts were added a few drops of 10% ferric chloride solution. Appearance of a green/yellow colour indicates the presence of tannins.

**Test for terpenoids: (Salkowiski's Test)**

Two ml of the extracts were mixed with 1 ml of chloroform and of conc. $H_2So4$ solution. A reddish brown colour at the inter phase indicates the presence of terpenoids.

**Phytochemical Profiling**

**Phytochemical profiling was done by High Performance Thin Layer Chromatography (HPTLC) methodology**

Since general phytochemical analysis was not sufficient enough to determine the type of phytoactive constituents, hence HPTLC was carried out. The chromatographic system used was HPTLC-DESAGA Applicator AS 30, 230 V, with HPTLC Densitometer CD 60, 230V, with Windows$^R$ software Pro Quant$^R$.

**Reagents and Chemicals**

Analytical grade methanol, toluene, ethyl acetate, chloroform, and formic acid were purchased from Qualigens Fine Chemicals, Mumbai, India. TLC plates used were Silica gel 60 $F_{254}$ plate of dimension 20 × 20 cm, 0.25 mm thickness manufactured by Merck KGaA, 64271 Darmstadt, Germany.

**High Performance Thin Layer Chromatography (HPTLC)**

Mobile phase developed for TLC separation of the root extracts were toluene–ethyl acetate–chloroform–formic acid (6:4:3:1) v/v, ethyl acetate-formic acid-glacial acetic acid-water (10:1:1:2) (v/v) and chloroform-glacial acetic acid-methanol-water (6:2:1:1) were used as mobile phase. A glass trough chamber (20 × 10 ×4 cm) was saturated for 20 min with the stated mobile phases before operating with each of the solvent systems separately. The standard and sample solutions were spotted manually as bands by using capillary tubes (length 4", bore 0.5 mm). HPTLC was performed on10 cm × 100 cm aluminium backed plates coated with 0.2-mm layers of silica gel 60 $F_{254}$ (Merck, Mumbai, India). Asiatic acid was used as a marker and applied on the plate as bands 8.0 mm wide, 6.0 mm apart, and 10.0 mm from the bottom edge of the chromatographic plate by using a DESAGA Applicator AS 30 sample applicator equipped with a 100-µL Hamilton (USA) syringe along with test sample. Ascending development to a distance of 80 mm was performed at room temperature (28 $\pm$2°C), with the above mentioned mobile phases, in a DESAGA glass twin-trough chamber previously saturated with mobile phase vapor for 20 min. After development, the plates were dried in air and then scanned at 240 nm (both the standards showed maximum absorption at this wavelength) with an HPTLC Densitometer CD 60, 230V, with Pro Quant software, using a deuterium lamp. Solutions of reference standards of markers (asiatic acid, 0.5 mg/mL; kaempferol, 0.5 mg/mL; b-sitosterol, 0.5 mg/mL; asiaticoside, 0.25 mg/mL; and mixture of methyl paraben and propyl paraben, 1 and 0.1 mg/mL) were used in the study. The markers were selected from the results of phytochemical analysis.

**Requisites for Studying Larvicidal Activity**

**Test mosquitoes**

*Aedes aegypti* larvae, which were derived from various places with clean stagnant water within and from south 24 parganas of West Bengal were colonized and maintained continuously for several generations since 2012 in a laboratory free of exposure to pathogens, insecticides, or repellents.

The laboratory colony was maintained at 25-30°C and 80-90% relative humidity under a photoperiod of 14:10 h (light/dark) in the dedicated insectary and quarantine facility maintained in the department of science and technology. Under these conditions, the full development from egg to adult lasted about 3-4 weeks. Larvae were fed on finely-ground bread crumbs.

**Larvicidal bioassay**

The larvicidal bioassay followed the WHO standard protocols (WHO 1981a) with slight modifications. For experimental treatment, one ml of *Centella asiatica* extract was dissolved in absolute ethanol was added to 224 ml of distilled water in a 500-ml enamel bowl, shaken to a homogeneous test solution. Then 25 early fourth instar larvae of *Ae. Aegypti* in 25 ml of distilled water were transferred to that bowl. Each experiment was performed in 5 replicates with a final total of 125 larvae for each concentration. The control solution was made with 1 ml of ethanol mixed with 249 ml of distilled water, while the untreated solution contained 250 ml of distilled water only. Symptoms of treated larvae were observed and recorded immediately and at timed intervals, and no food was offered to the larvae. Mortality and survival were registered after 24 h of the exposure period. The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality of each concentration. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. They might also show discoloration, unnatural positions, tremors, uncoordination, or rigor. All surviving larvae were separately reared and maintained at 25-30°C and 80-90% relative humidity in the insectary. Pupation and adult emergence of these mosquitoes were recorded. The assays were terminated 3 d after the last control mosquito emerged. The experiments were replicated four times.

**Data management and statistical analysis**

In this case where the control mortality (larvicidal) was between 5-20%, the observed percentage mortality (%M) was corrected by Abbott's formula (Abbott 1925):

Data for the anti-mosquito potential (larvicidal) were analyzed by means of computerized probit analysis (Harvard Programming; Hg1, 2), yielding a level of effectiveness at 50% and 95% mortality with 95% confidence intervals (95% C.I.).

## RESULTS AND DISCUSSION

**Phytochemical Analysis**

Phytochemical analysis of the root extracts of *Centella asiatic* L. showed the presence of flavanoids, glycosides, phenolics, saponins, steroids and terpenoids, but we found glycosides to be present in the acetone extracts also as mentioned in Table No.1.

**Optimization of Chromatographic Conditions**

Of the various combinations tried, finally a solvent system with ratio having chloroform-glacial acetic acid-methanol-water (6:2:1:1) gave the best resolution for asiatic acid after treatment with *P*-anisaldehyde. Measurements was done at 365nm as shown in Figure No.1. Markers have been used previously for the study.

Ethanolic extract of *Centella asiatica*, with a yield of 1.2 % (w/w), was semi-solid, greenish black residue and slightly aromatic. Larvicidal activity of this plant extract against fourth instar larvae of *Ae. Aegypti* is shown in Table No.2. The susceptibility of *Ae. Aegypti* to serial dilutions of the ethanol-extracted *Centella asiatica* was dose dependent[12]. Increasing the plant extract concentration from 60 to 200 mg/L increased the larval mortality range from 3 to almost 100%. High mortality (> 50% mortality) values were observed from 140 to 200 mg/L. No mortality was observed in control or untreated groups. Mortality of 96-100% was observed in the highest concentration, 200 ppm of the ethanol-extracted *Centella asiatica*. At the lowest concentration, 60 mg/L, there was very low mortality (3-4.5%). Many surviving larvae derived from the concentration of 60 mg/L failed to pupate and emerge as adults 3.8%. The ethanol-extracted

*Centella asiatica* showed promising larvicidal activity with $LD_{50}$ and $LD_{95}$ values of 140 and 200 mg/L, respectively. Observations carried out through the exposure period at room temperature revealed that immediately after exposure to ethanol-extract *Centella asiatica* solution, all larvae were still active and exhibited a normal appearance with the siphon pointed up and head hung down. The process of larval feeding, both collecting-filtering in the water column and collecting-gathering at submerged surfaces, were clearly seen. Between 5 and 10 min after treatment, some of the larvae became restless and frequently sank down and floated up quickly. At 15 min, the restlessness persisted, and tremor and convulsion at the bottom of the container were observed in approximately 2-3 larvae. Similar evidence of restlessness, tremors, and convulsions followed by paralysis was clearly seen at 45 min in approximately 4-5 larvae. At 1 h, approximately 1-2 moribund and dead larvae were found. For as long as 4 h after treatment, approximately one-third of the larvae were paralyzed and sank to the bottom of the bowl. More and more larvae exhibited toxic symptoms during 5 to 6 h. Subsequently, all of them died within 7 h in the 200 mg/L treatment. The ethanol-extracted *Centella asiatica* exhibited a delayed type of larval killing property because of non rapid mortality. The symptoms observed in treated larvae were similar to those caused by nerve poisons, i.e. excitation, convulsions, paralysis, and death.

| | | | | |
|---|---|---|---|---|
| | *% test mortality* | - | *% control mortality* | |
| %M = | (non-biting) | | (non-biting) | X 100 |
| | | | | |

100 - % control mortality
(non-biting)

**Table No.1: Phytochemical analysis of *C. asiatica* L. root extracts**

| S.No | Test | Solvents | | | |
|---|---|---|---|---|---|
| 1 | Alkaloids | **Methanol** | **Ethanol** | **Acetone** | **Chloroform** |
| | | - | - | - | + |
| 2 | Carbohydrates | - | - | - | - |
| 3 | Flavanoids | - | + | - | - |
| 4 | Glycosides | + | + | + | - |
| 5 | Phenolic Compounds | - | + | - | - |
| 6 | Proteins | - | - | - | - |
| 7 | Quinones | - | - | - | - |
| 8 | Saponins | + | + | - | - |
| 9 | Steroids | + | + | - | - |
| 10 | Tannins | - | - | + | - |
| 11 | Terpenoids | + | + | - | - |

**Table No.2: Larvicidal activity of the ethanol-extracts of *Centella asiatica* against fourth instar *Ae. Aegypti***

| S.No | *C.asiatic* (mg/L) | % Mortality (Mean ± SE) | Larvicidal activity (95% C.I., mg/L) | |
|---|---|---|---|---|
| 1 | 60 | 3.5±1.0 | $LD_{50}$ | $LD_{95}$ |
| | | | 140.0 | 200 |
| 2 | 70 | 5.0±2.2 | | |
| 3 | 80 | 11.8±1.5 | | |
| 4 | 90 | 15.0±12.8 | | |
| 5 | 100 | 20.2±7.4 | | |
| 6 | 110 | 24.2±2.4 | | |
| 7 | 120 | 35.5±1.0 | | |
| 8 | 130 | 43.2±2.4 | | |
| 9 | 140 | 57.2±2.0 | | |
| 10 | 150 | 67.6±1.5 | | |
| 11 | 160 | 77.4±2.3 | | |
| 12 | 170 | 81.2±1.5 | | |
| 13 | 180 | 86.3±1.2 | | |
| 14 | 190 | 92.5±1.6 | | |
| 15 | 200 | 96.1±1.3 | | |
| 16 | Control | 0 | | |
| 17 | Untreated | 0 | | |

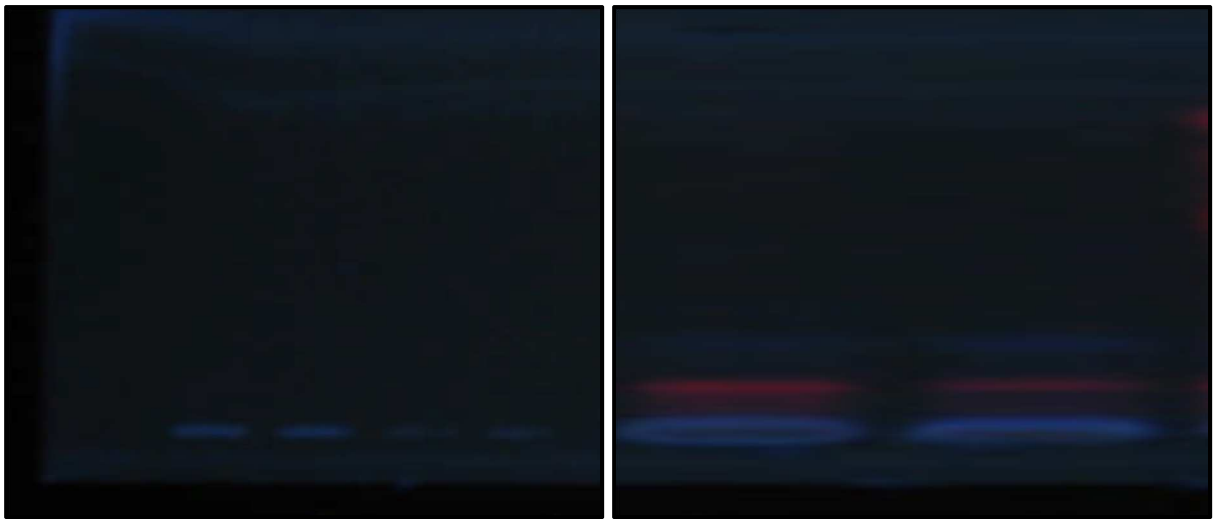

**Figure No.1: High Performance Thin Layer Chromatography (HPTLC) of the root extracts of *Centella asiatica* L. at 365 nm**

## CONCLUSION

In conclusion, *Centella asiatica* offers potential respite against *Ae. Aegypti*, particularly in its markedly larvicidal effect. Further studies of the active principles involved and their mode of action, formulated preparations for enhancing potency and stability, toxicity and effects on non-target organisms and the environment, and field trials are needed to recommend *Centella asiatica* as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

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**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

**BIBLIOGRAPHY**

1. Brinkhaus B, Lindner M, Schuppan D and Hahn E G. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*, *Phytomedicine,* 7(5), 2000, 427-448.
2. Mavondo G A, Mkhwananzi B N, Mabandla M V and Musabayane C T. Asiatic acid influences parasitaemia reduction and ameliorates malaria anaemia in P. berghei infected Sprague–Dawley male rats, *BMC Complementary and Alternative Medicine,* 16(1), 2016, 357.
3. Hendarto S K and Hadinegoro S R. Dengue encephalopathy, *Acta. Paediatr. Jpn,* 34(3), 1992, 350-357.
4. Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U and Thisyakorn C. Dengue infection: a global concern, *J. Med. Assoc. Thai,* 85(Supply 1), 2002, S25-33.
5. Granett P. Studies of mosquito repellents. II Relative performance of certain chemicals and commercially available mixtures as mosquito repellent, *J. Econ. Entomol,* 33(3), 1940, 566-572.
6. Karamchandani P V. Dengue group of fevers in India, *Lancet,* 19(1), 1946, (6386), 92.
7. Balaya S, Paul SD, D'Lima L V, Pavri K M. Investigations on an outbreak of dengue in Delhi in 1967, *Indian Journal of Medical Research*, 57(4), 1969, 767-74.
8. Chaturvedi U C, Mathur A, Kapoor A K, Mehrotra N K, Mehrotra R M L. Virological study of an epidemic of febrile illness with haemorrhagic manifestations at Kanpur, India, during 1968, *Bull World Health Organ,* 43(2), 1970, 289-93.
9. Parvez P, Ravi S, Majhi, Laserson K F, Narain J P, Chauhan H, Dhariwal A C and Chauhan L S. Dengue Fever Outbreak in Bardhaman District, West Bengal July-Aug 2013, *India EIS Conference November 2013),* 33.
10. Health on the March 2015-2016. State Bureau of Health Intelligence Directorate of Health Services, Government of West Bengal, 384.
11. Harborne J B. Phytochemical methods, London, *Chapman and Hills,* 1998, 286.
12. Choochote W, Tuetun B, Kanjanapothi D, Rattanachanpichai E, Chaithong U, Chaiwong P, Jitpakdi A, Tippawangkosol P, Riyong D and Pitasawat B. *Journal of Vector Ecology,* 29(2), 2004, 340-346.

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