

## Pharmacognostical and larvicidal evaluation of *Artocarpus lakoocha* Roxb. from Western Ghats

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Mosquitoes are the most important single group of insects acting as vector for many tropical and subtropical diseases. Using insecticides of plant origin to interrupt the life cycle, vector borne diseases can be controlled. *Artocarpus lakoocha* Roxb. popularly known as *Monkey Jack* or *Lakuch* is ethnobotanically used to treat fever, purge, skin ailments and also as an appetizer. The present study investigated the pharmacognostical and larvicidal activity of leaves and fruits of *A. lakoocha* against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae. As a step towards standardization of crude drug, the powder microscopy, physico-chemical analysis, TLC studies and HPTLC profiles of active pet ether extract were established. The larvicidal potential of successive solvent extracts were screened and expressed as LC<sub>50</sub> values. The pet ether extract of leaves and fruits were significant with LC<sub>50</sub> 241.36, 624.88, 1162.86 and 1180.95, 1286.69, 1398.69 µg/mL on the three species, respectively compared to other extracts. The results indicate that the triterpenoids and phytosterols present in the pet ether extract exerted a dose dependent larvicidal activity and the malarial vector, *An. stephensi* was more susceptible to the pet ether extract.

**Keywords:** *Artocarpus lakoocha* Roxb., *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, HPTLC, Mosquito larvicidal activity, Pharmacognostical evaluation.

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### Introduction

Natural products, especially from plants are becoming popular in developed and developing countries, due to herbal origin and lesser side effects. According to the World Health Organization, 65–80 % of the world's population relies up on the use of medicinal plants to derive primary health care benefits<sup>1</sup>. Industries manufacturing herbal drugs on large scale face problems relating to quality, authentication of crude drugs as well as standardization protocols for crude drugs, extracts, single and polyherbal formulations including quality control parameters. Thus to reach the global standards in herbal market, these problems needs to be addressed<sup>2</sup>. In practice, when plants become reliable sources for curing diseases, there arises need for development of systematic knowledge by

incorporating the features of identification, authentication, official standardization and then only of these herbal drugs should be utilized in formulations<sup>3</sup>.

Mosquitoes are the vectors for many dreadful diseases inflicting humans like malaria, dengue, yellow fever, west Nile encephalitis, lymphatic filariasis, dengue and chikungunya<sup>4</sup>. One of the effective methods to control these diseases is to target the vectors at all stages of the mosquito life cycle for interrupting the disease transmission<sup>5</sup>. Presently, the vector control efforts are focused on larval and adult stages.

*Artocarpus lakoocha* Roxb. commonly known called as *Monkey Jack* or *Lakucha* is an evergreen tropical timber yielding tree found in the high altitude regions of Himalayan range of India, Nepal, Bangladesh and China. It is also found invariably in the lower, middle and higher altitude of Western Ghats region<sup>6</sup>, from where the plant samples were collected. Wood, leaves, fruits and seeds of *A. lakoocha* are commonly used by the tribes and folk

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for fever, as appetizer and as aphrodisiac. In Bengal and among the *Mundas* of Chotanagpur, the seeds and the milk derived from seeds are popularly used as purge. The infusion of the bark has been used to treat skin ailments including sores and cracked skin<sup>7</sup>. In culinary practice, it is substituted for tamarind due to its sour and astringent properties<sup>8</sup>. The fine powder of bark is an excellent purulent. The tribals of Assam chew the bark, similar to betel nut and *Miji* tribes of Arunachal Pradesh use the tender leaves of *Lakucha* and *Mangifera indica* L. as a starter preparation for alcoholic beverages (locally called *Ongti*)<sup>9</sup>. Major phenolic compounds from the bark include lakoochins A and B, oxyresveratrol and galangin<sup>10,11</sup>. Other constituents include  $\beta$ -sitosterol, cycloartenol, cycloartenone,  $\alpha$ -amyrin acetate and lupeol acetate<sup>11,12</sup>.

The present study was undertaken to establish standardization profile and to investigate the larvicidal potential of the leaves and the fruits of *A. lakoocha* Roxb. against late 3<sup>rd</sup>/ early 4<sup>th</sup> instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.

## Materials and Methods

### Collection of plant materials

The leaves were collected during the flowering season (March to April, 2013) and fruits during the fruiting season (August to October, 2013) from the Western Ghats of Karnataka. The authentication of plant materials was done by Dr. Santhan P, Plant Taxonomist of Natural Remedies Pvt Ltd, Bengaluru and the herbarium specimen (223/2014 and 224/2014) were deposited in Natural Remedies Pvt Ltd, Bengaluru.

### Pharmacognostical evaluation

The plant materials were freed from extraneous matter, air-dried under shade, coarsely grounded, sieved to forty-mesh size and stored in airtight containers. Organoleptic investigations were carried out according to standard protocols<sup>13,14</sup>. For microscopic observations, the samples were preserved in 50 mL of 50 % ethanol : glacial acetic acid : formaldehyde (90:5:5). Microscopical sections and powder microscopy samples were previously boiled in chloral hydrate and treated with phloroglucinol-hydrochloric acid reagent and examined under microscope<sup>15</sup>. A systematic powder analysis of plant materials was also carried out<sup>16</sup>. Digital photomicrographs of sections and powder

characteristics were recorded under compound microscope with a built in analogue camera. The stomatal number and stomatal index, vein islet number and palisade ratio were determined using leaf samples treated with 5 % potassium hydroxide. The physico-chemical constants and fluorescence analysis<sup>17</sup> of the plant were done by using powder having mesh forty size.

### Extraction

Successive method of solvent extraction was carried out using non-polar to polar solvents- petroleum ether (Pet ether), chloroform and ethanol. The extracts were concentrated in rotavapor (Buchi) at 40 °C. The concentrated extracts were transferred in to glass vials and stored at ambient temperature. The color, consistency, nature and percentage yield of extracts were recorded. All the analyses were done in triplicate and the results were expressed as mean $\pm$ standard deviation. The preliminary phytochemical screening was carried out on all the extracts for the presence of different chemical constituents<sup>18</sup>.

### Preliminary screening for larvicidal activity

#### Vectors used

Larvae of *An. stephensi* (RS- Bangalore strain), *Ae. aegypti* (RS-Bangalore strain) and *Cx. quinquefasciatus* (RS-Bangalore strain) were obtained by culturing from cyclic colony maintained in the ACL class II insectory of National Institute of Malaria Research (NIMR-FU, ICMR), Bengaluru, India. The colony was kept free from exposure to pathogens, insecticides/repellents and maintained at 25–30 °C, 70 $\pm$ 5 % RH and a photo regime of 16 : 8 (L : D) h.

#### Culturing of larvae<sup>19</sup>

For culturing the colonies, eggs of *An. stephensi*, *Ae. aegypti* eggs and egg rafts (8–10) of *Cx. quinquefasciatus* were placed in separate enamel pans (30 x 25 x 4 cm) of 2 L capacity. Larvae were reared under a photoperiod of 14:10 (L:D) at 27 $\pm$ 2 °C and fed with powdered mixture of dog biscuits and dried yeast powder (3:1) and allowed to pupate. The pupae were collected from the pans, transferred into tap water and placed in screen cages (23 x 23 x 23 cm) where the adults emerged. The adults were provided with 10 % sucrose solution and on day 5 after emergence, females were allowed to feed on blood in membrane feeding apparatus. The late 3<sup>rd</sup>/ early 4<sup>th</sup> instar larvae from these colonies were used in the bioassays. Different concentrations of extracts

(10-3000 µg/mL) were prepared from the stock solution (10 mg/mL) by diluting with the tap water. The extracts were dissolved in 5 mL of DMSO and made up to 100 mL with distilled water with vigorous shaking. The stock solution was stored in capped volumetric flasks and covered with aluminum foil.

#### Larvicidal bioassay

All the extracts were tested against late 3<sup>rd</sup>/early 4<sup>th</sup> instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* for larvicidal bioassay according to the World Health Organization standard protocols<sup>19</sup>. Twenty five larvae of each vector species were transferred to tap water in sterile plastic bowls of 500 mL capacity. Test solutions of graded concentrations were prepared in triplicate with two replicas of control containing 1 mL of DMSO in 249 mL of tap water. Neemark<sup>TM</sup>, 50 ppm was used as positive control. All the bioassays were conducted at 27±3 °C, 85 % RH. The mortality was recorded after 24 h of the exposure period. Dead and moribund larvae were identified as when they failed to move or show characteristic diving reaction when probed with needle in siphon or cervical region or when the water was disturbed. The statistical data of larvicidal activity including LC<sub>50</sub>, LC<sub>90</sub>,  $\chi^2$  (heterogeneity) and fiducial limits were calculated by probit analysis using Statplus 2009 Professional software.

#### Chromatographic studies on pet ether extract

TLC profiles of all the extracts were developed by the method of optimization using different solvent systems to show maximum separation of phytoconstituents. Two mg of leaves and fruits extracts were dissolved in methanol and applied as thin bands on silica gel 60 F<sub>254</sub> (0.25 µ) pre-coated plates (Merck). The plates were developed in different solvent systems and air-dried. Visualization was done in day light (DL) and 254 nm. Later the plates were sprayed with vanillin-sulphuric acid (VS) and heated in hot air oven (maintained at 105 °C) for 15 minutes. The R<sub>f</sub> values were calculated and recorded<sup>20</sup>.

HPTLC fingerprint of the active pet ether extract of leaves and fruits were obtained by applying 10 µL on HPTLC plates (E. Merck, 10x10, 0.2 mm, Silica GF<sub>254</sub>) using CAMAG Linomat IV sample applicator (Muttentz, Switzerland) equipped with 100 µL Hamilton Syringe (Switzerland). The plates were developed in a twin trough chamber up to a distance of 9.0 cm in respective solvent systems. The developed plates were scanned at 254 nm by Camag TLC scanner

3.0 version and data was acquired. The plates were then derivatized with vanillin-sulphuric acid reagent, heated for 10 minutes at 105±2 °C and scanned under 254 nm.

## Results

#### Morphology and microscopy of leaves

The leaves were dark green with shiny ventral and pubescent dorsal surface. The leaf is pinnately compound 5-10-13 x 6-9-12 x 0.1-0.2 cm, alternate and oblong to ovate with cordate base and acute apex. The petiole is short and 1.2-1.9 x 0.3-0.2 cm. The margin is entire with reticulate venation. The odor is characteristic and taste is slightly bitter. The transverse section of leaves (Plate 1, hand drawn) exhibited the presence of long unicellular covering trichomes on upper and lower epidermis. Glandular trichomes with brown head and colorless stalk were found on lower epidermis. A single layer of chlorenchymatus palisade cells were present in upper epidermis and 3-5 layers of aerenchymatous mesophyll cells with chloroplast were present towards the lower epidermis. The midrib region contained numerous lignified and few unlignified fibers of 20-28-36 µm. The pith contained 8-10 vascular bundles arranged conjoint, collateral and open with metaxylem and medullary rays. Numerous anisocytic stomata were found, both in upper and lower epidermis. Stomatal number (154±3.0), stomatal index (19.4±1.22), vein islet number (36±0.63) and palisade ratio (2.1±0.3) were determined for the leaf.

#### Morphology and microscopy of fruits

The fruits were irregular, subglobose (6-9-10 x 5-7-9 x 4-6-8 cm) with pubescent outer surface. They

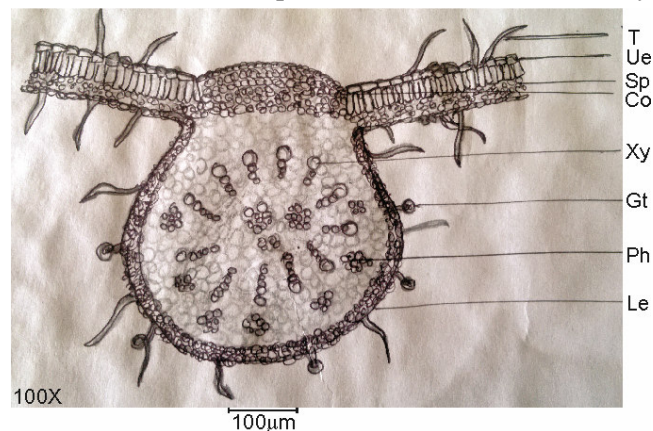


Plate 1—T.S. of leaf of *A. lakoocha* Roxb. (T-trichomes, Ue-upper epidermis, Sp- spongy parenchyma, Co- collenchymas, Xy- xylem vessels, Gt- glandular trichomes, Ph- phloem fibers, Le- lower epidermis)

appeared light yellow to green on outer surface and inner part was bright yellow colour with numerous seeds. The fruits had characteristic odor with sweet and sour, astringent taste. The transverse section of the treated dried fruits is presented in Plate 2 (hand drawn). The pericarp contained 10-14 layers of cells, epicarp 2-5 layers, mesocarp 5-6 layers and endocarp had about 5-8 layers. The cells of epicarp were polygonal with thin smooth cuticle. Mesocarp contained thick walled parenchymatous cells with starch grains and few tannin containing cells. Parenchymatous cells with numerous pits that are branched frequently were found along with lignified elongated fibers. The endocarp was lignified and

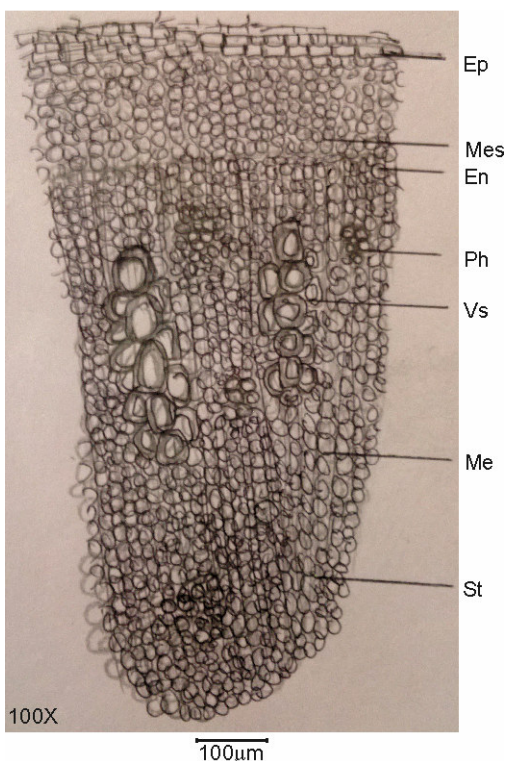


Plate 2—T.S. of fruit of *A. lakoocha* Roxb. (Ep-epicarp, Mes-mesocarp, En- endocarp, Ph- phloem fibers, Vs- vessels, Me-medullary, St- Starch)

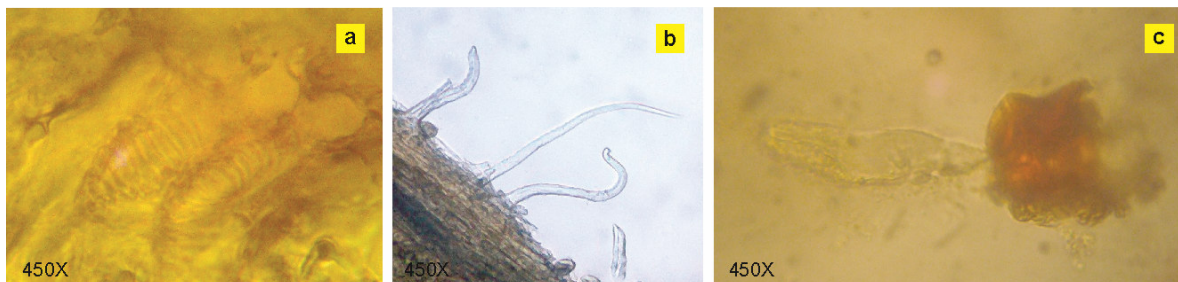


Plate 3—Powder microscopy of leaves of *A. lakoocha* Roxb. a) Lignified & unligified xylem vessels, b) Covering trichomes on upper epidermis and c) Glandular trichomes with brown head and colorless stalk

fibrous with tangentially elongated cells. Few rhombohedral crystals of calcium oxalate 0.3-0.4-0.5 µm were also found scattered in the endocarp.

#### Powder microscopy

##### Leaves

The leaf powder was olive green in color with characteristic odour and slight bitter in taste. The texture was coarse and fibrous. Microscopic observation revealed that lignified and unligified xylem vessels were present with spiral thickening along with pitted phloem fibers (Plate 3a-c). Long unicellular covering trichomes and glandular trichomes with brown head and colorless stalk were found attached to epidermal tissues.

##### Fruits

The dried powder of fruits appeared dark brown with characteristic odour and astringent sweet taste. The powder texture was rough. Numerous long transparent fibers, unligified phloem parenchyma along with lignified elongated fibers, single celled trichomes with thick cuticle and abundant starch granules with central hilum were found (Plate 4a-e).

#### Physico-chemical analysis

The values of the physico-chemical analysis of the powdered drug of leaves and fruits of *A. lakoocha* revealed that the drug complied with the quality control standards of herbal drugs (Table 1). For fluorescence analysis, drug powder was treated with different solvents like ethanol, methanol, water, etc. and observed under UV and ordinary light (Table 2). The color, consistency and percentage yield of successive solvent extraction are presented in Table 3. The phytochemical screening revealed the presence of proteins, alkaloids, glycosides, flavonoids, phenolics, sterols and triterpenoids in different extracts.

#### Larvicidal activity

The pet ether, chloroform and alcoholic extract of the leaves and fruits of *A. lakoocha* were tested for



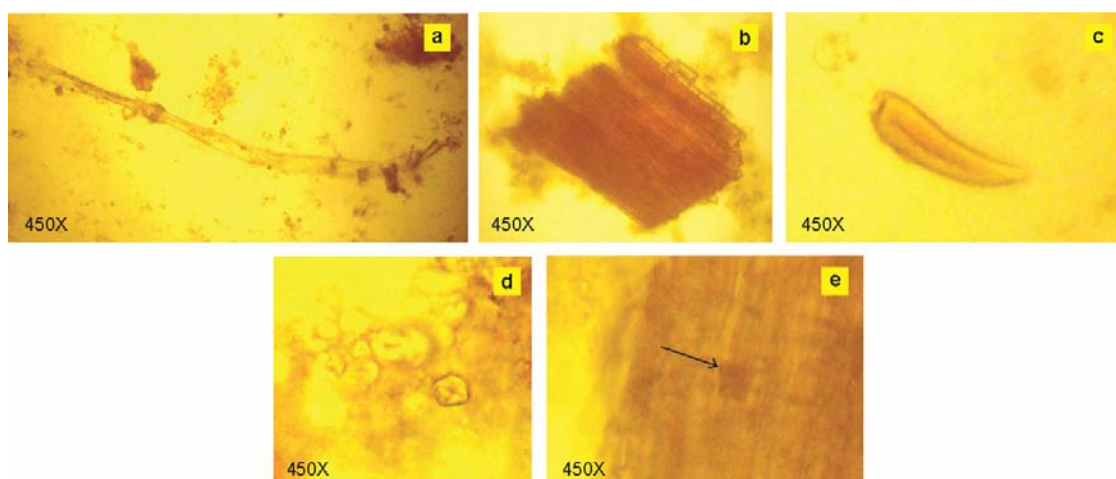


Plate 4—Powder microscopy of fruits of *A. lakoocha* Roxb. a) Transparent fibers, b) Pitted parenchyma, c) Covering trichomes with thick cuticle, d) Starch grains with central hilum and e) Rhombohedral Ca-oxalate

Table 1—Physico-chemical parameters of the leaves and the fruits of *A. lakoocha* Roxb.

Parameters (% w/w)	Plant Parts	
	Leaves*	Fruits*
Moisture content (LOD)	7.543 ± 0.310	10.040 ± 0.400
Pet ether soluble extractive value	6.452 ± 0.308	5.714 ± 0.630
Chloroform soluble extractive value	5.031 ± 0.004	9.583 ± 0.523
Ethanol soluble extractive value	5.990 ± 0.035	6.400 ± 0.510
Water soluble extractive value	10.865 ± 0.652	7.523 ± 0.576
Total ash	4.071 ± 0.159	3.553 ± 0.163
Water soluble ash	6.688 ± 0.26	10.248 ± 0.470
Acid insoluble ash	6.351 ± 0.180	0.970 ± 0.041
Foreign organic matters	0.200 ± 0.010	0.080 ± 0.010

\*Expressed as Mean ± Standard deviation

Table 2—Fluorescence analysis of the leaves and fruits of *A. lakoocha* Roxb.

Powder	Leaves			Fruits		
	Observation under			Observation under		
	WL	254nm	366nm	WL	254nm	366nm
as such	Green	Green	Black	Dark brown	Brown	Black
Water	Buff color	Green	Grey	Pale brown	Light yellow	Dark grey
H <sub>2</sub> SO <sub>4</sub>	Red	Yellow	Black	Black	Olive green	Black
1 N HCl	Pale buff	Yellow	Grey	Yellow	Bright yellow	Dark grey
Ethanol	Green	Yellow	Grey	Buff	Light grey	Light grey
Methanol	Olive green	Yellow	Grey	Pale yellow	Pale yellow	Dark grey
Me-NaOH	Yellow	Yellow	Grey	Yellow	Bright yellow	Dark grey
Et- NaOH	Pale yellow	Yellow	Grey	Light yellow	Light grey	Dark grey
5 % KOH	Brown	Brown	Black	Dark brown	Olive green	Black

WL-white light; HCl- hydrochloric acid; Me- methanol; Et- ethanol; NaOH- sodium hydroxide; KOH- potassium hydroxide

preliminary screening of larvicidal bioassay on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The pet ether extract of leaves showed LC<sub>50</sub> of 241.36, 624.88, 1162.86 µg/mL, chloroform extract showed LC<sub>50</sub> of 732.88, 1121.83, 1337.9 µg/mL and ethanolic

extract showed LC<sub>50</sub> of 1022.07, 1121.83, 3082.68 µg/mL on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively (Table 4). Among all the extracts tested, the pet ether extract of leaves showed larvicidal activity. The order of activity based on LC<sub>50</sub>

values of the leaves was found to be pet ether extract > chloroform extract > ethanolic extract with  $p < 0.05$ . The pet ether extract of fruits showed larvicidal activity with lesser  $LC_{50}$  of 1180.95, 1286.69, 1398.69  $\mu\text{g/mL}$  on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively compared to chloroform and ethanolic extract (Table 4). For the fruits, the order of activity was found to be pet ether extract > ethanolic extract > chloroform extract ( $p < 0.05$ ) on the three species of mosquitoes. The results revealed that the

pet ether extracts of leaves and fruits have good larvicidal potential compared to other two extracts.

While comparing pet ether extract of leaves with that of fruits, the larvicidal effects were more profound in the leaves with lower  $LC_{50}$  values. Many botanical extracts have shown comparable  $LC_{50}$  values as that of the pet ether extracts of leaves and fruits<sup>22</sup>. The phytochemical screening of pet ether extracts of leaves and fruits revealed the presence of phytosterols and triterpenoids, which may have exerted the larvicidal activity.

Table 3—Physical properties of the leaves and the fruits extracts of *A. lakoocha* Roxb.

Extract	Color	Consistency	Extractive Value* (% w/w)
Leaves of <i>A. lakoocha</i> Roxb.			
Pet ether	Green	Oily, sticky	6.351 $\pm$ 0.430
Chloroform	Green	Solid mass	3.362 $\pm$ 0.238
Ethanol	Brown	Viscous, sticky	4.862 $\pm$ 0.521
Fruits of <i>A. lakoocha</i> Roxb.			
Pet ether	Pale yellow	Oily, sticky	5.732 $\pm$ 0.120
Chloroform	Brown	Viscous	2.195 $\pm$ 0.331
Ethanol	Brown	Sticky	3.865 $\pm$ 0.256

\*Expressed as Mean  $\pm$  Standard deviation

#### Chromatographic studies of leaves and fruits

TLC analyses of successive solvent extracts of both the leaves and the fruits were carried out by solvent optimization. For leaves, pet ether extract gave  $R_f$  values as that of HPTLC analysis in toluene : ethyl acetate (8:2). The chloroform extract in methanol : chloroform (8:2) gave  $R_f$  of 0.83, 0.92 in DL and 0.76, 0.81, 0.83, 0.97 after spray with VS. Ethanolic extract in ethylene chloride : methanol (8.5:1.5) gave  $R_f$  values of 0.44, 0.67 in DL, 0.20, 0.67 in UV and 0.20, 0.23, 0.44, 0.55, 0.67 after derivatization. TLC analysis of pet ether extract of fruits in methanol: formic acid (6:0.4) revealed constituents similar to

Table 4—Preliminary screening of larvicidal bioassay of *A. lakoocha* Roxb.

Vector species	Extract\	$LC_{50}$ Values $\mu\text{g/mL}$	$LC_{90}$ Values $\mu\text{g/mL}$	$\chi^2$	Limits for $LC_{50}$ $\mu\text{g/mL}$	
					LCL	UCL
Leaves of <i>A. lakoocha</i> Roxb.						
<i>An. stephensi</i>	Pet ether	241.36	2714.44	6.98	177.41	312.34
	Chloroform	732.88	4467.56	4.40	349.41	1199.61
	Ethanol	1022.07	1549.27	1.48	932.76	1106.46
<i>Cx. quinquefasciatus</i>	Pet ether	624.88	2994.99	68.45	268.78	1452.73
	Chloroform	1121.83	5339.85	16.67	470.24	3224.44
	Ethanol	1231.36	4675.60	35.83	539.22	2046.18
<i>Ae. aegypti</i>	Pet ether	1162.86	1548.82	2.65	1,130.85	1191.48
	Chloroform	1337.95	2377.36	1.16	1217.05	1461.99
	Ethanol	3082.68	5613.25	6.04	2922.43	3253.95
Fruits of <i>A. lakoocha</i> Roxb.						
<i>An. stephensi</i>	Pet ether	1180.95	1359.12	11.06	1145.55	1223.74
	Chloroform	11824.42	25755.86	7.15	11501.43	13225.55
	Ethanol	1503.74	1850.49	8.80	483.79	525.36
<i>Cx. quinquefasciatus</i>	Pet ether	1286.69	1549.52	1.62	1232.16	1341.00
	Chloroform	10527.91	32605.55	1.70	9078.36	12839.84
	Ethanol	1650.81	1940.66	35.25	1592.75	1717.01
<i>Ae. aegypti</i>	Pet ether	1398.69	1658.52	1.62	1322.16	1356.00
	Chloroform	11282.12	28555.13	2.62	10231.69	12342.79
	Ethanol	1944.32	2615.15	3.72	1738.42	2179.64

$LC_{50}$ - Lethal concentration at which 50 % larvae are dead,  $LC_{90}$ - Lethal concentration at which 90 % larvae are dead,  $\chi^2$ - Chi-square values, LCL- Lower confidence limit, UCL-Upper confidence limit ( $p \leq 0.05$ )

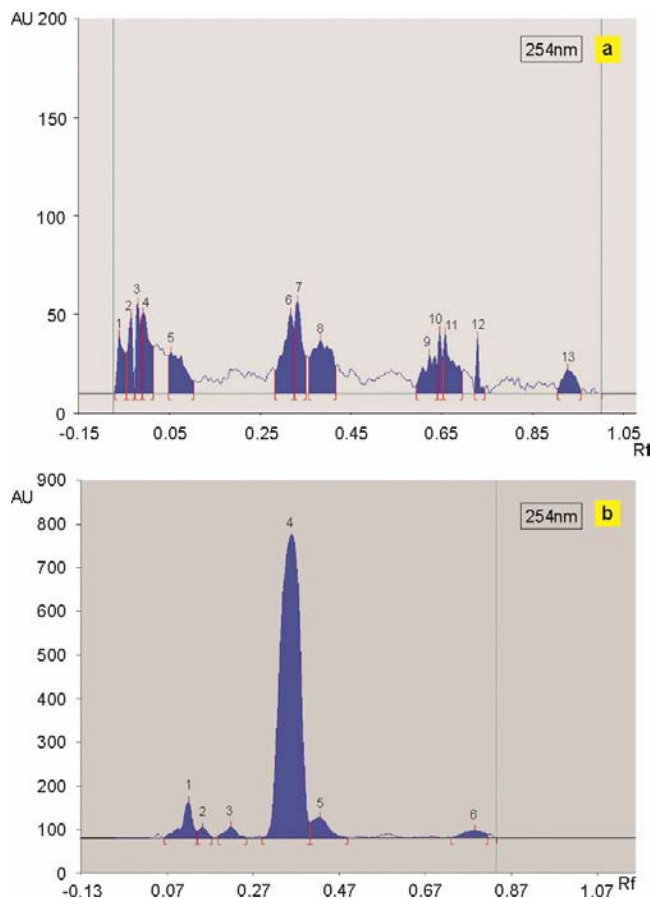


Plate 5—HPTLC chromatogram. a) Pet ether extract of leaves and b) Pet ether extract of fruits of *A. lakoocha* Roxb. at 254 nm

that of HPTLC profile. The chloroform extract of fruit in methanol : chloroform (7:3) gave R<sub>f</sub> of 0.89 in DL, 0.74, 0.85 in UV and 0.74, 0.85, 0.87, 0.89 after spray with VS. The ethanolic extract of fruit in pet ether: ethyl-acetate (9:1) gave R<sub>f</sub> values of 0.27, 0.52 in DL, 0.52, 0.8 in UV and 0.27, 0.52, 0.69, 0.8 after spray with VS.

The HPTLC studies of pet ether extract of the leaves in toluene : ethyl acetate (8:2) revealed the presence of 3 spots of R<sub>f</sub> values 0.68, 0.74, 0.87 in DL and 11 spots of R<sub>f</sub> values 0.02, 0.06, 0.18, 0.36, 0.40, 0.49, 0.52, 0.68, 0.73, 0.74, 0.87 were visualized under UV 254 nm. Thirteen spots with R<sub>f</sub> values of 0.02, 0.06, 0.18, 0.28, 0.36, 0.40, 0.49, 0.52, 0.68, 0.73, 0.74, 0.87, 0.92 were derived after spraying with VS (Plate 5a). HPTLC of pet ether extract of fruits in methanol : formic acid (6:0.4) R<sub>f</sub> values of 0.09, 0.21, 0.37 were observed DL and two spots with R<sub>f</sub> values 0.37, 0.40 were seen under UV light 254 nm. After derivatization, 6 spots with R<sub>f</sub> values of 0.09, 0.13, 0.21, 0.37, 0.40, and 0.78 were obtained (Plate 5b). The chromatographic analysis revealed presence of

greater number of constituents in the leaves than that of the fruits.

## Discussion

In traditional systems of medicine, plants are the major source of crude drugs<sup>21</sup>. The standards are required for confirmation of identity of the plant and to ascertain the quality of drugs. According to World Health Organization, quality control of herbal drugs, pharmacognostical parameters are the first step towards establishing its identity and purity that distinguishes the plant from other species<sup>3</sup>. *A. lakoocha* has several uses in traditional systems of medicine and is ethanobotanically important. Hence, in the present study the pharmacognostical investigation of leaves and fruits of *A. lakoocha* was taken up as a step towards standardization of crude drug. The physico-chemical analysis of the powdered drugs complied with the quality control standard for medicinal plants.

Mosquitoes are responsible for transmission of more diseases than any other group of arthropods. Mosquito control is warranted due to resistance, stagnant development of newer insecticides and their adverse effects on environment. Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future, as they are relatively safe, biodegradable and are readily available in many areas of the world. Many common plants like *Azadirachta indica*, *Ocimum* species, *Mentha piperita*, *Eucalyptus camaldulensis*, *Eucalyptus urophylla* have been reported for their larvicidal potential against various species of mosquitoes<sup>22</sup>. The leaves and seeds extract of *Melia azadirachta* L. evaluated for larvicidal activity by Senthilnathan *et al.*<sup>23</sup> Showed 96 % mortality at a concentration of 2 %.

In the present study, the pet ether, chloroform and alcoholic extract of the leaves and fruits of *A. lakoocha* were tested for larvicidal activity. The pet ether extract of leaves and fruits revealed good larvicidal activity at LC<sub>50</sub> of 241.36, 624.88, 1162.86 µg/mL and 1180.95, 1286.69, 1398.69 µg/mL on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. The non-polar pet ether extract of both leaves and fruits showed highest activity compared to chloroform and alcoholic extracts. A comparative study carried out on the three *Ocimum* species with different solvents extracts by Rajamma *et al.*<sup>24</sup>, showed that the pet ether of *O. basilicum* had better larvicidal effects compared to chloroform and ethanolic and aqueous extracts at low LC<sub>50</sub> values of 39.31 µg/mL. Similarly, Maheshwaran *et al.*<sup>25</sup> tested the pet ether, chloroform

and ethanolic extracts of *Leucas aspera* L. and found that the larvicidal activity was more profound in the pet ether extracts than the other two solvent extracts. The results of present study comply with these studies. Shailendra *et al.*<sup>26</sup> reported the larvicidal activity of methanolic extracts of *A. lakoocha* fruit pericarp on larval stages of *Ae. aegypti* at 100 mg/mL (100000 µg/mL) on the 3<sup>rd</sup> day of observation. In the present study, 100 % mortality of the pet ether extracts of leaves and fruits of *A. lakoocha* was found at concentrations less than 2000 µg/mL on all the three species of mosquitoes which is the advantage of successive method of extraction. Among the leaves and the fruits of *A. lakoocha*, the leaves were more effective as larvicidal agent compared to that of fruits with LC<sub>50</sub> values of 241.36, 624.88, 1162.86 µg/mL on *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Markouk *et al.*<sup>27</sup> reported the larvicidal activity of the latex, flowers and leaves of *Calotropis procera* Wild., seeds and leaves of *Solanum sodomaeum* L., *Cotula cinerea* L. and berries of *Solanum elaeagnifolium*. Among the tested plants, higher larvicidal effect was revealed by the latex extracts of *Cx. procera* and the berries of *S. elaeagnifolium* at concentrations lesser than 325 µg/mL.

TLC profiles of successive solvent extracts were established which have not been reported previously. Hence, TLC profiles were developed to separate the phyto-constituents. Focusing on larvicidal potential of pet ether extracts, HPTLC fingerprints were done to quantify the phyto-constituents. Of the leaves, the prominent peak 3 and 7 showed highest absorbance with peak areas of 26.43 and 28.54 mAU, respectively, which may be responsible for the larvicidal activity. The HPTLC fingerprint of pet ether extracts can be utilized for quantification of formulations with *A. lakoocha*. The result of the study has shown that pet ether extracts of the leaves of *A. lakoocha* have promising mosquito larvicidal activity. Further, a bioassay-guided chemical fractionation protocol to identify the components responsible for the larvicidal activity and development of herbal pesticidal formulation is under way.

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#### References

- 1 Muniappan A and Ignacimuthu S, Ethno botanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India, *J Ethnopharmacol*, 2011, **134**(3), 851-864.
- 2 Harish P, Meeting report- Herbal Drugs, *Curr Sci*, 2001, **81**(1), 15.
- 3 Vandana G, Virjidhar, Anupam S and Rohit D, Facts about standardization of herbal medicine: A review, *J Chinese Integrat Med*, 2012, **10**(10), 1077-1082.
- 4 Anjali R, Anupam G and Goutam C, Mosquito larvicidal potential of four common medicinal plants of India, *Indian J Med Res*, 2014, **140**, 102-108.
- 5 Hardin J and Jackson F, Applications of natural products in the control of mosquito transmitted diseases, *African J Biotechnol*, 2009, **8**(25), 7373-7378.
- 6 Khare C P, Indian Medicinal Herbs: An illustrated dictionary, Springer-Verlag, Berlin/ Heidelberg, 2007, 66.
- 7 Kirtikar K R and Basu B D, Indian medicinal plants, ICMR, New Delhi, 1935, **3**, 2588.
- 8 Sarala P and Krishnamurthy S R, Monkey jack: underutilized edible medicinal plant. Nutritional attributes and traditional foods of Western Ghats, Karnataka, India, *Indian J Tradit Know*, 2013, **13**(3), 629-634.
- 9 Shrivastava, K, Greeshma A G and Srivastava B, Biotechnology in Tradition-Methods of starter preparation for alcoholic beverages employed by different tribal communities of Arunachal Pradesh, *Indian J Tradit Know*, 2012, **11**(1), 194-201.
- 10 Anonymous, Wealth of India: Raw materials and Industrial products, CSIR, New Delhi, 1985, **1**, 453- 455.
- 11 Puntumchai A, Kittakoop P, Rajviroongit S, Vimuttipong S, Likhitwitayawuid K and Thebtaranonth Y, Lakoochins A and B, new antimycobacterial stilbene derivatives from *Artocarpus lakoocha*, *J Nat Prod*, 2004, **67**(3), 485-486.
- 12 Chauhan J S and Kumari G, New glycoflavonol from the root bark of *Artocarpus lakoocha*, *Planta Medica*, 1979, **37**(9), 86-88.
- 13 World Health Organization, Quality control methods for medicinal plant materials, WHO /PHARM/ 92.559, 1998, 04-46.
- 14 Anonymous, Indian Pharmacopoeia, Vol 2., Ministry of Health and Family welfare, Government of India, Controller of publications, New Delhi, 1996, A-53-54, 95, 97, 109.
- 15 Iyengar M A and Nayak S G, Anatomy of crude drugs, Manipal Press Limited, Manipal, 1997, 01-08.
- 16 Iyengar M A, Pharmacognosy of powdered crude drugs, Manipal Press Limited, Manipal, 1997, 21-31.
- 17 Madhavan V, Priyanka G, Gurudeva M R and Yoganasimhan S N, Pharmacognostical studies on root of



- Nothosaerva brachiata* wt, - A botanical source of ayurvedic drug, Pashanabheda, *Indian J Tradit Know*, 2010, **9**(4), 629-634.
- 18 Harborne J B, Phytochemical methods, Chapman and Hall Ltd, London, 1973, 49-188.
- 19 Guidelines for laboratory and field testing of mosquito larvicides, WHO Pesticide Evaluation Scheme WHO/CDS/WHOPES/GCDPP/2005, WHO, Geneva, 2005, 13, 7-12, 32-33.
- 20 Wagner H, Baldt S, Plant drug analysis A thin layer chromatography atlas, Springer-Verlag, Berlin Heidelberg, 1996, 349-352.
- 21 Bigoniya P, Singh C S, and Shukla A, Pharmacognostical and physicochemical standardization of ethnopharmacologically important seeds of *Lepidium sativum* Linn. and *Wrightia tinctoria* R. Br. *Indian J Nat Prod Resour*, 2011, **2**(4), 464-471
- 22 Shaalan E A S, Canyon D, Younes M W F, Abdel W H and Mansour A H, A review of botanical phytochemicals with mosquitocidal potential, *Environ Int*, 2005, **31**, 1149-1166.
- 23 Senthilnathan S, Savitha G, George D K, Narmadha A, Suganya L and Chung P, Efficacy of *Melia azadirachta* L. extract on the malarial vector *Anopheles Stephensi* Liston (Diptera: Culicidae), *Biores Technol*, 2006, **97**, 1316-1323.
- 24 Rajamma A J, Sonal D, Sateesha S B, Tiwari S N and Ghosh S K, Comparative larvicidal activity of different species of *Ocimum* against *Culex quinquefasciatus*, *Nat Prod Res*, 2011, **25**(20), 1916-1922.
- 25 Maheswaran R, Sathis S and Ignacimuthu S, Larvicidal activity of *Leucus aspera* (Willd.) against the larvae of *Culex quinquefasciatus* Say. and *Aedes aegypti* L., *Int J Integrat Biol*, 2008, **2**(3), 214-217
- 26 Shailendra K M B, Rakesh K M C, Bharath A C, Vinod K H R, Prashith K T R, Nandini K C, Rakshitha M N and Raghavendra H L, Screening of selected biological activities of *Artocarpus lakoocha* Roxb. (Moraceae) fruit pericarp, *J Basic Clin Pharm*, 2010, **1**(4), 239-245.
- 27 Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek H B and Jana M, Evaluation of some Moroccan medicinal plant extracts for larvicidal activity, *J Ethnopharmacol*, 2000, **73**, 293-297.