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STUDY OF BIO-LARVICIDAL ACTIVITY ON AEDES AEGYPTI THROUGH BIOASSAY BY CRUDE EXTRACT OF DIFFERENT PARTS OF CARICA PAPAYA PLANT ALONG WITH AN IN-SILICO APPROACH FOR TOXICITY AND MUTAGENICITY OF ESTABLISHED PHYTOCHEMICALS BY USING QSAR MODELLING

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ABSTRACT

Carica papaya Linn is a common tree, and containing phytocompounds for medicinal and bio-larvicidal properties. The objective of the present study was to detect percentage mortality of larvae of *Aedes aegypti* by aqueous extract of different parts such as leaf, seed, unriped fruit, latex and flower of *C. papaya* and *in silico* predictive study for toxicity and mutagenicity of established phytochemicals of *C. papaya* through QSAR modelling. Bioassay experiment was done with different parts of aqueous extract with following dilutions viz. 20%, 40%, 60%, 80% and 100% respectively and % mortality was observed in *A. aegypti* for 24h and 48h durations. Next, QSAR modelling was done to detect toxicity in *D. magna*, *P. promelas*, rat oral and mutagenicity for established phytochemicals and the confirmation of phytochemicals was done by TLC and NMR. The present study revealed that phytochemicals of *C. papaya* leaf, seed and unriped fruit extract have highest ability to destroy mosquito larvae of *A. aegypti* compared to latex and flower extract. Also, QSAR modelling revealed phytocompounds such as Benzylisothiocyanate, Quercetin, Hentriacontane, Carpaine, and Linalool showed higher toxicity in *D. magna* while in *P. promelas* highest toxicity found in this manner as Linalool >Benzylisothiocyanate> Carpaine>Quercetin>Hentriacontane. It was also observed that two phytocompounds such as Benzylisothiocyanate and Quercetin were mutagenic positive others found negative.

Quercetin was confirmed both TLC and NMR study. In conclusion, studied phytochemicals from crude extracts can be isolated and prepared bio-larvicide from each phytocompound prior to functional assay.

KEYWORDS: *Carica papaya*; Phytochemicals; Bioassay of extracts; Predictive toxicity and mutagenicity; QSAR modelling; *In silico* study.

INTRODUCTION

Mosquito-borne diseases can be controlled by killing of mosquito larvae at source The destroy of larvae is done by using larvicides. The synthetic larvicides have potent impact on environment especially toxicity to aquatic biota.[1-4] For this reason, researchers have developed larvicides from plant derived phytochemicals.[5-10] However, it was known that few phytochemicals are also toxic, mutagenic and carcinogenic to living organisms and human.[11]

Among various plant species, different parts of *Carica papaya* plant is well known biolarvicide when larvae contact with crude extract.[7,10,12-13] Moreover, it is an important task to know the exact phytochemical is acting as toxin in the crude extract of different parts of *C. papaya*. In this context, researchers have established mathematical model as quantitative structure activity relationship (QSAR) to screen large numbers of compounds and the predictive results for toxins help in functional assay in future.[14-24] The bacterial mutagenicity test to know whether the compound is mutagenic positive or negative.[25] According to Lipnick,[26] determination of aquatic toxicity by structure-activity relationships help to detect easily the environmental fate and effects on biota.

The present study was aimed to detect percentage mortality of larvae of *Aedes aegypti* by aqueous extract of different parts such as leaf, seed, unriped fruit, latex and flower of *C. papaya* in the laboratory condition as well as prediction of toxicity and mutagenicity of established phytochemical of C. papaya by using QSAR modelling software.

MATERIALS AND METHODS

Papaya plant sample collection

The papaya plant (*C. papaya* Linn.) parts samples were collected from the college campus, Serampore, West Bengal, India. The sample collection area was selected as per no air and water pollution and the latitude 22^{o} 45′ N and longitude 88^{o} 21′ E respectively.

Preparation of aqueous extracts

The aqueous extract of different parts of papaya (*C. papaya*) were prepared by using fresh leaves, unriped fruits, seeds, flowers and latex (Fig. 3). The extraction was done by the method of WHO[27] and Chandrasekaran et al.[10] with some modifications. All the parts were cleaned by keeping with the running tap water, followed by distilled water, then kept on the blotting paper to soak the excess water. Each part of 10 nos. were kept in mortar and macerated by pastel along with dechlorinated tap water. The solution was filtered and taken in a clean glass bottle as a stock solution (100%). The organic solvents were not used.

Toxicity test for larvae of *Aedes aegypti*

From this stock solution, different dilutions were prepared as 20%, 40%, 60%, 80% and 100%. The supplied larvae (*A. aegypti*) were kept in the aerated water prior to toxicity test and 10 nos. were used in each petri dish as per higher to lower dilutions (100% - 20%). The test was performed twice as replicate. The percentage mortality was recorded in each dilution for 0hr, 24hr and 48hr. The percentage mortality was calculated by using following formula:
Mortality (%) = No of larvae Dead / No. of larvae×100

Phytochemicals screening through Thin Layer Chromatography and NMR

The chamber for chromatography partition was filled by a little amount with the mobile solution of n-butanol: acetic acid: water (BAW) in the ratio of 4:1:5 was kept for 1 hour for saturation of the chamber as per protocol by Nugroho et al.[28] with slight modifications. The solution to be tested was placed on a Whatman chromatographic paper and a line was drawn through the spot to mark the "baseline". A sample "frontline" was marked at distance of 10cm from the baseline. The chromatographic paper was dried at 40^{o}C for 10 minutes in hot air oven. On the base line the aqueous extract of leaf was spotted using a capillary tube and allowed to dry at 30^{o}C for 2 minutes. The dried paper was then further placed in the chamber and the bottom edge was dipped into the mobile phase. After the mobile phase moved upto the sample from the paper was further dried. The paper was further sprayed using the solution of Antimony III chloride and chloroform to develop the spot material, which was migrated from original sample spot. The paper was observed under 360nm (UV rays). The Rf value was calculated by the method of Daody et al.[29] by using following formula:
Rf = distance from the baseline to the spot / distance from the baseline to the solvent front

The NMR was performed in leaf to detect the phytochemical and the molecular structure was identified.

Phytochemicals selection for QSAR modelling

In the present study, the selection of phytochemicals in the different parts such as leaf, seed, unriped fruit, latex and flower of *C. papaya* were based on available literature study.[30-31]

Predictive toxicity study through QSAR modelling

The prediction of toxicity as the LC_{50} values in cladocera, *Daphnia magna* and fathead minnow (cyprinid fish), *Pimephales promelas*, LD_{50} value of oral exposure in rat and mutagenicity positive or negative were obtained by using the software T.E.S.T (Toxicity Estimation Software Tool), Version 4.1.[21] All the data were obtained by consensus method, which is basically the average predicted LC_{50} and LD_{50} values simulated from average value as per QSAR methodologies. The predicted value for each chemical was obtained within the software after statistical interpretation as correlation coefficient (R^2) value to know the level of significance of the predictive value.

Statistical analysis

Statistical analysis was done to determine correlation coefficient value (R^2) for the percentage dilution versus percentage mortality by plotting a regression curve in each case of experimental set and analysed two durations (24hr and 48hr) of exposure and their replica. In the present study, R^2 value for all four experimental sets were determined through software (Microsoft 10, Excel 2016, add-on statistical toolpack).

RESULTS AND DISCUSSION

Bioassay of crude extracts of different parts of *C. papaya*

Bioassay experiment with special reference to toxicity test were performed on the larvae of *Aedes aegypti* after acute exposure to different parts such as leaf, seeds, unriped fruit, latex and flower aqueous extracts of *C. papaya*. The acute exposure was performed 24hr and 48hr duration for each plant part after preparation of percentage dilution such as 20%, 40%, 60%, 80% and 100% in aqueous medium. The experiment was carried out duplicate and the average value was taken for percentage mortality curve. 24hr recorded average percentage mortality i.e. 30%, 55%, 80%, 85% and 100% in leaf extract, 20%, 45%, 55%, 65% and 80% in seed extract, 0%, 25%, 50%, 65% and 85% in unriped fruit extract, 30%, 55%, 80%, 85% and 100% in latex extract and 0%, 0%, 0%, 5% and 15% in flower extract having 20%, 40%, 60%, 80% and 100% dilution while the 48hr recorded average percentage mortality i.e. 55%, 70%, 80%, 95% and 100% in leaf extract, 30%, 55%, 75%, 85% and 95% in seed extract, 10%, 30%, 65%, 80% and 90% in unriped fruit extract and 0%, 0%, 10%, 20% and 30% in

flower extract having percentage of 20, 40, 60, 80 and 100 dilutions (Figs 1-5). The correlation coefficient (R^2) values were observed for leaf, seed, unriped frut, latex and flower 95% and 98%, 96% and 95%, 99% and 96%, 75% and 87% and 72% and 94% for 24hr and 48h duration respectively. The dose-response curve is exhibited in Figs 1-5.

This mosquito species spread the parasites of Dengue fever and is a potent threat worldwide.[6,32-34] The well-known synthetic compounds are used to eradicate the larval population of mosquito to prevent human from mosquito-borne diseases.[1] But there is a possibility of ecotoxicological impact on aquatic biota by several synthetic pyrethroids.[2-4,35] The present study revealed that phytochemicals of *C. papaya* leaf, seed and unriped fruit extract has highest ability to destroy mosquito larvae of *A. aegypti* as biolarvicidal agents or can be used as biolarvicide for mosquito control compared to latex and flower extract (Figs 1-5), which has evidenced with other reports.[6-7,10,12] According to Sesanti et al.,[36] seed extract is more toxic than leaf extract in mosquito larvae (*Anopheles* sp.) but in present study higher toxicity was observed in the extract of unriped fruit followed by seed and leaf of *C. papaya* (Figs 1-3). Moreover, Malathi and Vasugi[7] documented that the extract of plant parts of *C. papaya* showed toxicity variation on ethanol and aqueous medium to mosquito larvae (*A. aegypti*).

TLC and NMR of phytochemicals in leaf of *C. papaya*

In Table 6, the Rf value was obtained highest for Quercetin (0.66), followed by Kaempferol (0.64) and Myricetin 3-rhamnoside (0.45), which are confirmed as flavonoids (Fig 6). The NMR study was revealed that the phytocompound is Quercetin and the molecular structure is exhibited in Fig 7. Interestingly, TLC revealed that three flavonoids such as Quercetin, Kaempferol and Myricetin 3-rhamnoside were obtained as per Rf value (Table 6 and Fig. 24). This result supported by Canini et al.[30] that *C. papaya* leaf contains Quercetin and NMR study is confirmed the presence of Quercetin. Therefore, it is suggesting further study should be carried out with other organic solvents to more effective biolarvcide on *A. aegypti* especially with latex and flower.

QSAR modelling for predictive toxicity and mutagenicity

The selection of phytochemicals for predictive toxicity screening was done based on highest percentage mortality in larvae by leaf, seeds and unriped fruit extract of *C. papaya*. In Table 1, among 22 phytocompounds, the CAS no. was obtained only for 13 phytochemicals. It was also noted as NF (not found) for six phytochemicals due to unavailability of validated

database in the T.E.S.T. software and the predictive toxicity data were obtained only for six compounds.

In case of *D. magna*, lowest LC_{50} value (ppm) was obtained for Benzylisothiocyanate (0.26), followed by Quercetin (0.53), Hentriacontane (0.61), Carpaine (1.69), Linalool (1.77) and highest was found in Malic acid (220.53) followed by Citric acid (68.06) respectively. In case of *P. promelas*, lowest LC_{50} value (ppm) was obtained for Hentriacontane (0.0008), followed by Quercetin (0.78), Carpaine (0.99), Benzylisothiocyanate (7.75), Linalool (9.24) and highest was found in Malic acid (690.09) followed by Citric acid (453.47) respectively. In case of oral exposure in rat, lowest LD_{50} value (ppm) was obtained for Benzylisothiocyanate (131.59), followed by Carpaine (1598.82), Linalool (2054.27), Malic acid (2600.88), Quercetin (2639.57), Citric acid (3469.87) and highest was found in Hentriacontane (10660.07) respectively. In case of mutagenicity prediction, all above mentioned phytocompounds such as Hentriacontane (-0.06 -ve), Linalool (0.03 -ve), Malic acid (0.10 -ve), Citric acid and Carpaine (0.24 -ve) were obtained mutagenic negative except Benzylisothiocyanate (0.50 +ve) showed as positive (Table 1). In Table 2, the prediction based on statistical analysis in context to correlation coefficient (R^2) values were obtained for each phytocompound in each studied organism. In case of *D. magna*, the R^2 value was obtained 96% for Carpaine, 77% for Benzylisothiocyanate, 92% for Hentriacontane and Citric acid, 89% for Linalool, 87% for Malic acid and 90% for Quercetin respectively. In case of *P. promelas*, the R^2 value was obtained 81% for Carpaine, 69% for Benzylisothiocyanate, 74% for Hentriacontane, 87% for Linalool, 86% for Citric acid, 81% for Malic acid and 89% for Quercetin respectively. In case of rat, the R^2 value was obtained 92% for Carpaine, 87% for Benzylisothiocyanate, 93% for Hentriacontane, 77% for Linalool, 74% for Citric acid, 83% for Malic acid and 78% for Quercetin respectively. In Table 3, the prediction based on statistical analysis in context to concordance, sensitivity and specificity values were obtained for each phytocompound. The values for concordance, sensitivity and specificity were obtained as 100%, 100% and 100% for Carpaine, 97%, 100% and 96% for Benzylisothiocyanate, not found any value for Hentriacontane, 100% in all for Linalool, 97%, 100% and 95% for Citric acid, 90%, 62% and 100% for Malic acid and 83%, 91% and 67% for Quercetin respectively.

The predictive toxicity in *D. magna*, *P. promelas* and oral rat and Ames mutagenicity of different phytocompounds found in several parts of *C. papaya* to know impact on aquatic and

terrestrial biota when exposed to biolarvicide Among 20 phytochemicals, only 6 phytocompounds able to test in the T.E.S.T. software due to availability of CAS no. in the chemical database. The predictive toxicity study showed toxicity on arthropod by the exact phytocompound and *A. aegypti* belongs to arthropod. The present *in silic*o screening can be helpful to study for making biolarvicide after isolation of these phytocompounds such as Benzylisothiocyanate, Quercetin, Hentriacontane, Carpaine, and Linalool due to highest toxicity in *D. magna* while highest toxicity was obtained in this manner as Linalool > Benzylisothiocyanate > Carpaine > Quercetin > Hentriacontane in *P. promelas*. It was also known in present predictive study that two phytocompounds such as Benzylisothiocyanate and Quercetin were observed mutagenic positive but other phytocompounds were mutagenic negative (Table 1). The TLC and NMR study revealed that Quercetin is confirmed in the leaf of *C. papaya*, which has evidenced with other report.[28]

Interestingly, several researchers have reported the toxicity on *A. aegypti* by using crude extract as biolarvicide.[6-7,10,12] but the present *in silico* approach may be useful to know the exact phytocompound, which can be used as biolarvicide after isolation from the parts of *C. papaya*. The predictive toxicity and mutagenicity study by using T.E.S.T. software, which has developed by USEPA,[21] and major *in silico* screening through QSAR modelling of different synthetic as well as natural compounds for larvicides have been well-established by several researchers.[37-38]

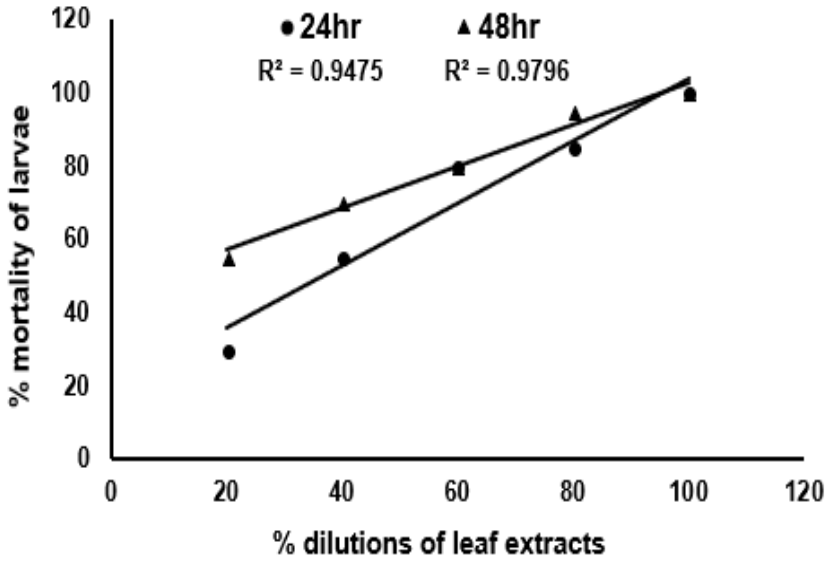

Fig. 1: Dose-response curve for 24hr and 48hr duration on larvae of *A. aegypti* exposed to leaf extracts of *C. papaya*.

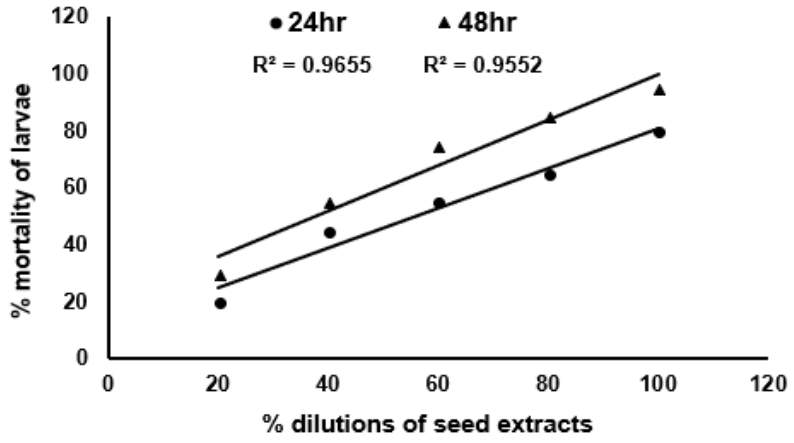

Fig. 2: Dose-response curve for 24hr and 48hr duration on larvae of *A. aegypti* exposed to seed extracts of *C. papaya.*

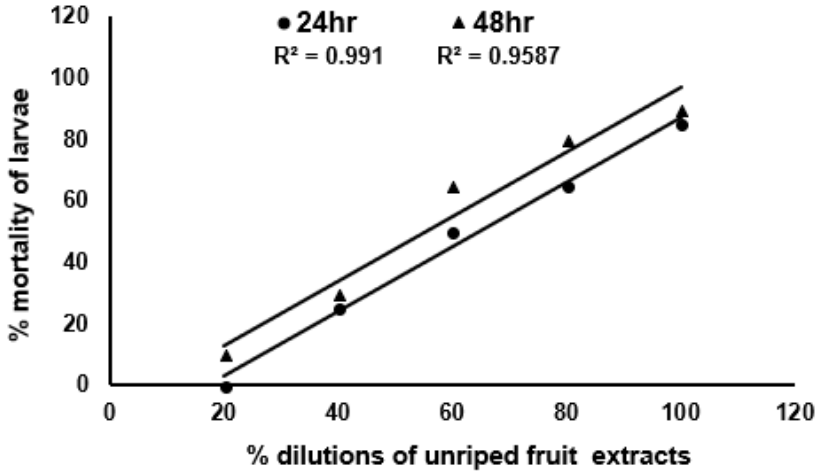

Fig. 3: Dose-response curve for 24hr and 48hr duration on larvae of *A. aegypti* exposed to unriped fruit extracts of *C. papaya*

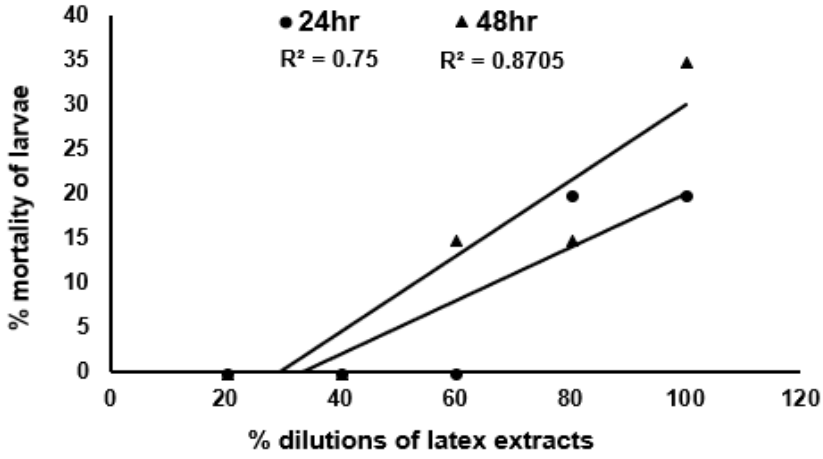

Fig. 4: Dose-response curve for 24hr and 48hr duration on larvae of *A. aegypti* exposed to latex extracts of *C. papaya.*

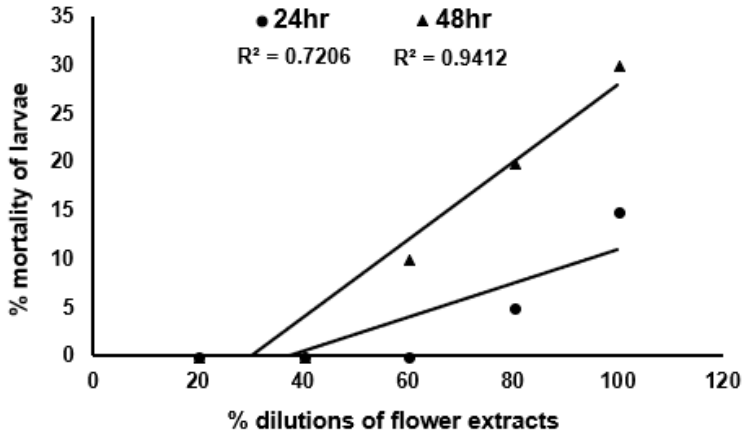

Fig. 5. Dose-response curve for 24hr and 48hr duration on larvae of *A. aegypti* exposed to flower extracts of *C. papaya.*

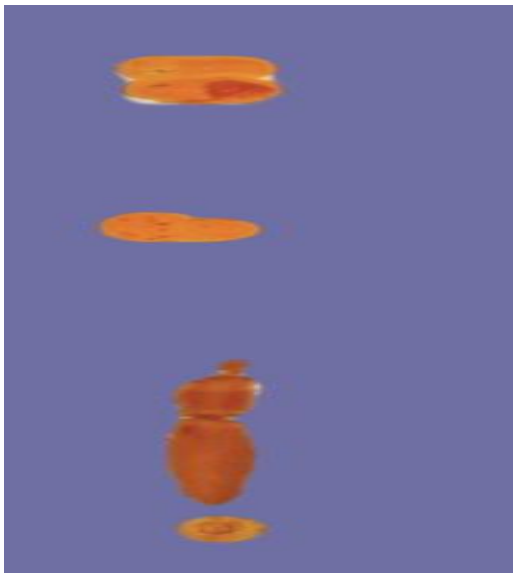

Fig. 6: TLC plate observed under UV light (360nm).

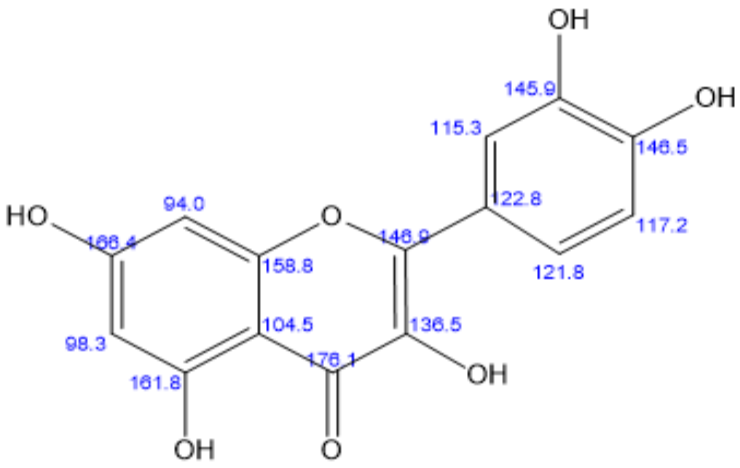

Fig. 7: Two-dimension structure of isolated compound in leaf of *C. papaya*.

Table 1: Predictive toxicity (LC_{50} and LD_{50} values) estimation through QSAR modelling in different organisms exposed to available phytochemicals.

Sl. No.	Phytochemicals	*Daphnia magna* LC_{50} (ppm)	*Pimephales promelas* LC_{50} (ppm)	Oral rat LD_{50} (ppm)	Ames mutagenicity
1.	Carpaine	1.69	0.99	1598.82	0.24 (-ve)
2.	Pseudocarpaine	NF	NF	NF	NF
3.	Dehydrocarpaine I	NF	NF	NF	NF
4.	Dehydrocarpaine II	NF	NF	NF	NF
5.	Choline	NF	NF	NF	NF
6.	Carposide	NF	NF	NF	NF
7.	Benzylisothiocyanate	0.26	7.75	131.59	0.50 (+ve)
8.	β-sitosterol	NF	NF	NF	NF
9.	Hentriacontane	0.61	0.0008	10660.07	-0.06 (-ve)
10.	Linalool	1.77	9.24	2054.27	0.03 (-ve)
11.	Citric acid	68.06	453.47	3469.87	0.24 (-ve)
12.	Malic acid	220.53	690.09	2600.88	0.10 (-ve)
13.	Quercetin	0.53	0.78	2639.57	0.55 (+ve)

Table 2: Correlation coefficient (R^2) value in different organisms for each phytocompound after simulation through QSAR modelling.

Sl. No.	Phytochemicals	*Daphnia magna* (R^2) value	*Pimephales promelas* (R^2) value	Oral rat (R^2) value
1.	Carpaine	96%	81%	92%
2.	Pseudocarpaine	NF	NF	NF
3.	Dehydrocarpaine I	NF	NF	NF
4.	Dehydrocarpaine II	NF	NF	NF
5.	Choline	NF	NF	NF
6.	Carposide	NF	NF	NF
7.	Benzylisothiocyanate	77%	69%	87%
8.	β-sitosterol	NF	NF	NF
9.	Hentriacontane	92%	74%	93%
10.	Linalool	89%	87%	77%
11.	Citric acid	92%	86%	74%
12.	Malic acid	87%	81%	83%
13.	Quercetin	90%	89%	78%

Table 3: Prediction statistics of mutagenicity for each phytocompound after simulation through QSAR modelling.

Sl. No.	Phytochemicals	Concordance	Sensitivity	Specificity	No. of chemicals
1.	Carpaine	1.000 (30 out of 30)	1.000 (6 out of 6)	1.000 (24 out of 24)	30
2.	Pseudocarpaine	NF	NF	NF	NF
3.	Dehydrocarpaine I	NF	NF	NF	NF
4.	Dehydrocarpaine II	NF	NF	NF	NF

5.	Choline	NF	NF	NF	NF
6.	Carposide	NF	NF	NF	NF
7.	Benzylisothiocyanate	0.967 (29 out of 30)	1.000 (7 out of 7)	0.957 (22 out of 23)	30
8.	β-sitosterol	NF	NF	NF	NF
9.	Hentriacontane	NF	NF	NF	NF
10.	Linalool	1.000 (30 out of 30)	1.000 (6 out of 6)	1.000 (24 out of 24)	30
11.	Citric acid	0.967 (29 out of 30)	1.000 (8 out of 8)	0.955 (21 out of 22)	30
12.	Malic acid	0.900 (27 out of 30)	0.625 (5 out of 8)	1.000 (22 out of 22)	30
13.	Quercetin	0.829 (29 out of 35)	0.913 (21 out of 23)	0.667 (8 out of 12)	35

CONCLUSION

It is concluded from the present bioassay results that extracts of leaf, seeds and unriped fruit of *C. papaya* can be used as biolarvicide for mosquito *A. aegypti*. It is well-established that the extracts of *C. papaya* plant parts are suitable for biolarvicide in several reports.[6-7,10,12] but researchers have observed separately leaf or seed or latex or root extracts in different species of mosquito. Herein, present study is based on toxicity assay in *A. aegypti* after exposed to different plant parts of *C. papaya* and confirmed extracts of leaf, seeds and unriped fruit of *C. papaya* showed highest toxicity in *A. aegypti*. It is suggesting in future study that single or combination of phytochemicals present in *C. papaya* can be screened to detect inhibitory activity of each phytocompound on acetylcholinesterase enzyme of mosquito. It is also important to detect phytochemicals toxicity in other aquatic macroinvertebrates and fish species because this biolarvicide exposed to aquatic ecosystem when it use for mosquito killing. An *in silico* work for established phytochemicals of *C. papaya* plant parts is confirming for biolarvicide.[6-7,10,12] but the predictive acute toxicity study, the LC_{50} values of few phytocompounds were obtained toxic in *D. magna* and *P. promelas* as well as 1 compounds was showed mutagenic while all were less toxic to rat by oral exposure (LD_{50} values). All the predictive toxicity and mutagenicity data were studied through QSAR modelling software (T.E.S.T.) recommended by USEPA.[21] Major research works have been carried out by using crude extracts of different parts of *C. papaya* as biolarvicide but in aquatic ecosystem impact on other organisms are lacking Although, individual phytochemical such as alkaloid, lignin, phytosterol, stanol etc have studied toxic, carcinogenic and mutagenic to the biota.[11,39-40] The present QSAR modelling study may support in future to know the mechanisms of toxicity and mutagenicity for each natural

chemical, which is found in combined form phytochemicals in extract of different parts of *C. papaya* for biolarvicide.

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CONFLICT OF INTEREST

Authors declare none.

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