ISSN: 2331-5083

Advances in Zoology and Botany

Volume No. 13
Issue No. 3
September - December 2025

ENRICHED PUBLICATIONS PVT. LTD

JE-18, Gupta Colony, Khirki, Extn, Malviya Nagar, New Delhi-110017
PHONE : - + 91-8877340707
E-Mail : info@enrichedpublications.com

ISSN: 2331-5083

Advances in Zoology and Botany

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ISSN: 2331-5083

Advances in Zoology and Botany

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ISSN: 2331-5083

Advances in Zoology and Botany

(Volume No. 13, Issue No. 3, Sep - Dec 2025)

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ISSN: 2331-5083

Reproduction Traits of the Native Chicken Variety Maintained at College of Poultry Production and Management, Hosur

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ABSTRACT

The reproduction parameters of the Native chicken variety were maintained at College of Poultry Production and Management (CPPM), Hosur and were studied at Poultry Farm Complex (PFC), Veterinary College and Research Institute, Namakkal during the period between April 2020 and April 2021. The reproduction parameters like Hen day egg production (HDEP), Hen housed egg production (HHEP), Hen housed hatching egg production (HHHEP), Fertility and Hatchability were studied. The Native chicken variety attained sexual maturity (149 days) much earlier than other native chicken in India. The results of hen housed egg production (No.) were 40.0±0.45 and 66.6±0.60 up to 40th and 52nd week, respectively. Fertility (per cent) of the Native chicken variety ranged between 93.1±0.21 and 95.9±0.32 with an average of 94.68 per cent. The recorded hatchability (TES) up to 52 weeks had ranged between 82.8±0.55 and 88.1±0.07 with an average hatchability of 85.70 per cent. The reproduction traits were higher than many other indigenous chicken with mean hatchability of 85.0 per cent, fertility of 94.8 per cent and production of 45.6 chicks per hen during the production period of 52 weeks.

Keywords Native Chicken, Hen Day Egg Production, Hen Housed Egg Production, Fertility and Hatchability

1. Introduction

Poultry farming is one of the fast-growing industries in India in which the organized sector of poultry industry is contributing nearly 67 per cent of the total output and the rest 33 per cent by the unorganized sector. The total egg production in India from commercial poultry is 95.17 billion and backyard poultry is 19.21 billion contributing 83.20 per cent and 16.80 per cent of the total production of eggs, respectively [1]. Native chickens are reared in a free-range extensive system with very little input in the form of grain or farm by-products and contribute to the unorganized sector in India. The average productive output of native chicken is very low, with 60 - 70 eggs per bird per annum.

Faruque et al. [2] stated that the indigenous chicken populations have the privilege of superiority over exotic chicken breeds due to several desired characteristics like broodiness, self-defense from predators, adaptability to adverse environments, disease resistance, lesser health care requirements,

characteristic taste and flavour of the meat, brown shelled eggs, rich in threonine and valine and a better price for the indigenous poultry products. Assefa and Melesse [3] found that the indigenous chicken contributes high quality animal protein in the form of eggs and meat for home consumption as well as for sacrifices and are also easily managed by all even the poorest of the poor including women and children. The demand for indigenous chicken is very high since their products are more preferred in comparison to commercial poultry due to better flavour, lower cholesterol content and higher amino acids (Threonine and Valine) in the eggs and lean as well as pigmented meat rich in amino acids (Arginine and Lysine). Indigenous chicken breeds are pushed to extinction because of commercialization of poultry systems and the lack of breeding programmes to improve the production potential. In view of the importance of indigenous poultry breeds under backyard production systems, breeding strategies to improve the productivity of native chicken should be considered without compromising their native characteristics such as hardiness, better immune status, flight, broodiness, etc. However, consumer preferences at market are dictated by brown shell colour and small size egg, etc. The native chicken variety is distributed to the farming community of the area and is well received by the community for backyard rearing. The Native chicken variety attained sexual maturity (149 days) much earlier than other native chicken in India. The results of hen housed egg production (No.) were 40.0±0.45 and 66.6±0.60 up to 40th and 52nd week, respectively. The reproduction traits were higher than many other indigenous chicken with mean hatchability of 85.0 per cent, fertility of 94.8 per cent and production of 45.6 chicks per hen during the production period of 52 week.

2. Research and Methodology

A Sum of 60 male birds and 460 female birds (out of 540 growers of each sex) were selected and reared in four replicates of 15 male and 115 female birds with a mating ratio of 1:8 in each replicate under a deep litter system Poultry Farm Complex, Veterinary College and Research Institute, Namakkal and the parameters like Hen day egg production, Hen housed egg production, Fertility and Hatchability were studied from 21-52 weeks of age. A sex separated feeding was followed during the breeding period (21-52 weeks of age) in which males were given with male breeder diet (ME 2604 kcal/kg and crude protein 16 per cent) in height adjusted feeder and females were given female breeder diet (ME 2635 kcal/kg and crude protein 17 per cent) based on nutrient specifications for layer breeder chicken. The experimental birds were protected and maintained under standard vaccination and bio-security protocols.

2.1. Production Traits

2.1.1. Body weight and Body Weight Gain

Individual sex wise body weights (g) were recorded at weekly intervals up to 52 weeks of age by using an electronic weighing balance nearest to 1.0 g accuracy.

2.2. Reproduction Traits

2.2.1. Feed Consumption and Feed Conversion Ratio

Feed consumption (g) was recorded at weekly interval up to 52 weeks of age and FCR for female pullets were calculated in terms of FCR per kg egg mass and dozen eggs during laying period. FCR was calculated as follows:

$$\text{i) FCR/ kg egg mass} = \frac{\text{Kg of feed consumed}}{\text{Kg of egg produced}}$$

$$\text{ii) FCR/ dozen eggs} = \frac{\text{Amount of feed consumed (g)}}{\text{No. of dozen Egg}}$$

2.2.2. Age at Sexual Maturity/Age at First Egg

Age at sexual maturity of a bird was recorded in days when the first egg was laid. The average period in days from the date of hatch to the date of the first egg laid was calculated as age at sexual maturity and was expressed in days.

2.2.3. Body Weight at Sexual Maturity

The body weight (g) of the bird was recorded soon after the first egg was laid. The birds were weighed on a single pan balance to the accuracy of one gram.

2.2.4. Egg Production

During the experimental period, the egg production was recorded daily. Based on the data, egg production was calculated in terms of weekly hen day (per cent) and cumulative hen housed (number) egg production. Sample egg weight was recorded at weekly intervals.

2.2.4.1. Rate of Lay

The continuous laying of eggs without pause on a particular day of production and Hen day egg production is calculated as follows:

$$\text{Hen day egg production (\%)} = \frac{\text{Rate of lay (\%)}}{\text{Number of hens present in particular day}} \times 100$$

2.2.4.2. Peak Production

The highest per cent of egg production was achieved at particular week, followed by a decrease in subsequent week.

2.2.4.3. Age at 50 % Production

The age at which the flock reaches 50 % of their egg production from its total egg produced.

2.2.5. Fertility and Hatchability

The reproductive performance of native chicken under an intensive system of rearing was studied from 21 to 52 weeks of age. All settable eggs obtained in a week were incubated at weekly intervals. All the

settings were analysed to record the average fertility and hatchability of the total egg set (TES) and fertile egg set (FES) and were calculated.as follows:

$$\text{i) Fertility (\%)} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set for incubation}} \times 100$$

$$\text{ii) Hatchability(TES)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of eggs set for incubation}} \times 100$$

$$\text{iii) Hatchability(FES)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of fertile eggs set for incubation}} \times 100$$

3.Statistical Analysis

The data on egg production, fertility and hatchability were analysed using the descriptive method of statistical analysis.

4.Results and Discussion

4.1. Body Weight at Breeder Phase

The mean (±S.E.) body weight (g) of the Native chicken variety maintained at CPPM, Hosur from 21 to 52 weeks of age is presented in Table 1. The body weight of male and female at 40th week was 2570.3±15.34 and 1726.18±15.01g, respectively. The body weight of male and female at 52nd week was 2709±7.21 and 1883.43±8.04 g, respectively. The average weekly body weight gain during the period ranges between 20 and 40 g with an average gain of 25 g in male, ranges between 15-20 g with an average gain of 17 g in females.

The Native chicken variety of CPPM has a higher body weight than native chicken of Jharkhand [4] and native chicken variety of Belagaum division of Karnataka [5] at the 32nd week of production, Aseel chicken at the 40th week of age [6] and the 48th week of age [7], native chicken variety of Belagaum division of Karnataka at 52nd week [5] and falls within the range of findings of Qureshi et al. [8] in Aseel chicken.

4.2. Feed Efficiency in Laying Phase

The mean (±S.E.) weekly and cumulative feed efficiency per dozen eggs and per kg egg mass of Native chicken variety maintained at CPPM, Hosur from 21 to 52 weeks and is presented in Table 2 and Table 3, respectively. The feed efficiency per dozen eggs ranged between 6.5±0.28 and 4.6±0.14 from the 30th week to the 52nd week of age. Similarly, the feed efficiency per kg egg mass ranged between 12.1±0.68 and 7.4±0.20 from the 30th week to the 52nd week of age.

3

Table 1. Mean (±S.E.) body weight (g) of Native chicken variety

Age	Male (n)	Female (n)
21st week	1881.0±78.09 (47)	1346.02±16.16 (126)
22nd week	2097.0±27.83 (39)	1415.06±16.71 (102)
23rd week	2179.2±32.50 (37)	1475.08±16.70 (114)
24th week	2269.9±25.79 (27)	1527.36±13.88 (106)
25th week	2319.7±23.08 (32)	1571.55±16.34 (118)
26thweek	2349.1±24.78 (36)	1576.04±13.59 (118)
27th week	2417.8±15.37 (31)	1604.22±12.86 (97)
28th week	2421.0±24.91(38)	1606.26±12.02 (110)
29th week	2426.6±96.36 (27)	1641.61±14.81 (72)
30th week	2434.0±19.87 (30)	1662.11±14.72 (99)
31stweek	2475.2±16.98 (33)	1670.39±11.44 (111)
32ndweek	2481.6±73.82 (36)	1676.36±12.23 (115)
33rdweek	2494.1±16.61 (32)	1690.54±12.37 (123)
34th week	2516.2±14.27 (33)	1697.85±13.57 (114)
35th week	2529.0±16.76 (35)	1706.60±15.94 (114)
36th week	2539.7±20.75 (37)	1707.34±13.42 (123)
37th week	2546.4±16.81 (34)	1720.45±11.32 (103)
38th week	2551.9±26.10(35)	1720.05±11.63 (122)
39th week	2557.2±16.96 (33)	1721.78±16.09 (103)
40th week	2570.3±15.34 (59)	1726.18±15.01 (440)
41st week	2572.4±17.93 (35)	1738.49±20.25 (121)
42nd week	2577.4±23.40 (37)	1753.28±11.92 (112)
43rd week	2581.1±15.56 (33)	1757.69±12.04 (118)

44th week	2584.1±20.65 (36)	1762.64±13.93 (103)
45th week	2589.7±14.57 (31)	1776.88±17.02 (122)
46th week	2595.2±19.69 (37)	1789.90±12.20 (101)
47th week	2601.5±23.27 (33)	1796.54±16.08 (107)
48thweek	2630.1±20.10 (37)	1814.88±10.22 (116)
49th week	2646.0±13.21 (34)	1850.96±19.62 (114)
50th week	2674.4±19.37 (37)	1868.69±11.29 (107)
51st week	2686.2±20.39 (34)	1881.05±10.55 (118)
52nd week	2709.1±7.21(59)	1883.43±8.04 (437)

(n)- Number in parentheses indicate the number of observations

Table 2. Mean (±S.E.) feed efficiency (per dozen of egg) of Native chicken variety

Age	Feed efficiency per dozen egg (n=4)	
	Weekly	Cumulative
24th week	0.0±0.00	0.0±0.00
25th week	9.2±0.45	31.5±3.87
26thweek	5.0±0.19	16.2±1.18
27th week	3.9±0.13	11.0±0.50
28th week	3.7±0.19	8.7±0.37
29th week	3.4±0.16	7.4±0.30
30th week	3.2±0.17	6.5±0.28
31stweek	3.3±0.04	6.0±0.21
32ndweek	3.7±0.18	5.7±0.20
33rd week	3.7±0.17	5.4±0.20
34th week	3.7±0.14	5.3±0.19
35th week	3.8±0.15	5.1±0.19
36th week	3.8±0.16	5.0±0.18
37th week	3.8±0.16	4.9±0.18
38th week	3.9±0.15	4.9±0.18
39th week	4.0±0.11	4.8±0.17
40th week	4.0±0.10	4.7±0.17
41st week	4.0±0.11	4.7±0.16
42nd week	4.1±0.12	4.7±0.16
43rd week	4.1±0.09	4.6±0.16
44th week	4.2±0.08	4.6±0.15
45th week	4.2±0.10	4.6±0.15
46th week	4.2±0.12	4.6±0.15

Table 3. Mean (±S.E.) feed efficiency (kg/kg) of native chicken variety

Age	Feed efficiency per kg egg mass (n=4)	
	Weekly	Cumulative
24th week	0.0±0.00	0.0±0.00
25th week	20.0±1.84	66.7±10.03
26thweek	10.2±0.55	32.9±2.95
27th week	7.8±0.27	22.0±1.42
28th week	7.1±0.40	16.9±0.98
29th week	6.3±0.35	13.8±0.73
30th week	6.0±0.38	12.1±0.68
31stweek	6.1±0.11	11.0±0.52
32ndweek	6.3±0.32	9.8±0.39
33rd week	6.4±0.31	9.4±0.38
34th week	6.4±0.22	9.0±0.34
35th week	6.4±0.25	8.8±0.32
36th week	6.4±0.25	8.5±0.31
37th week	6.5±0.26	8.4±0.31
38th week	6.6±0.24	8.3±0.30
39th week	6.8±0.17	8.2±0.29
40th week	6.8±0.15	8.1±0.28
41st week	6.8±0.16	8.0±0.28
42nd week	6.9±0.19	7.9±0.27
43rd week	7.1±0.13	7.9±0.26
44th week	7.1±0.13	7.9±0.26
45th week	7.0±0.16	7.7±0.26
46th week	7.0±0.19	7.5±0.26

46th week	4.2±0.12	4.6±0.15
47th week	4.3±0.10	4.6±0.15
48th week	4.4±0.13	4.6±0.14
49th week	4.5±0.13	4.6±0.14
50th week	4.6±0.11	4.6±0.14
51st week	4.7±0.07	4.6±0.14
52nd week	5.2±0.30	4.6±0.14

n - Number of observations from four replicates

46th week	7.0±0.19	7.5±0.26
47th week	7.0±0.16	7.4±0.26
48th week	7.1±0.23	7.4±0.25
49th week	7.3±0.21	7.4±0.25
50th week	7.4±0.15	7.4±0.24
51st week	7.5±0.10	7.4±0.20
52nd week	8.4±0.45	7.4±0.20

n - Number of observations from four replicates.

The feed efficiency per dozen eggs observed in the present study is better than native chicken variety of Mysore division of Karnataka [9], in native chicken variety of Bangalore division of Karnataka [10], native chicken of Tiruvannamalai [11] and native chicken variety of Belagaum division of Karnataka [5] and the feed per kg of egg mass is better than native chicken in Tiruvannamalai was observed by Balamurugan et al. [11].

4.3. Age at Sexual Maturity / Age at First Egg

The age at sexual maturity / age at first egg of the Native chicken variety maintained at CPPM, Hosur in the present study was recorded as 149 days of age. The population of the Native chicken variety attained 5 per cent hen day egg production at 165 days of age.

The age at sexual maturity of the Native chicken variety observed in the study coincides with Miri chicken in Umiam [12], Aseel chicken in Bangladesh [13, 14] and TANUVAS Aseel chicken [15]. The age at sexual maturity is earlier than Aseel chicken [12], native chicken in Andhra Pradesh [16] and Aseel chicken in Haryana [17].

4.4. Body Weight at Sexual Maturity

The mean (±S.E.) body weight of the female sex of the Native chicken variety maintained at CPPM, Hosur in the present study at sexual maturity was observed as
1438±14.06 g.

Table 4. Mean (±S.E.) Reproductive performance of Native chicken

Age	Egg weight in gram (n)	HDEP in per cent	HHEP in No.	HHHEP in No.
21st week	0.00±0.00	0.0±0.00	0.0±0.00	0.0±0.00
22nd week	33.6±2.89 (7)	0.2±0.07	0.0±0.00	0.0±0.00
23rd week	38.3±2.80 (34)	1.3±0.28	0.1±0.01	0.0±0.00
24th week	38.8±1.42 (123)	5.1±0.54	0.3±0.04	0.0±0.00
25th week	39.7±2.03 (94)	15.4±0.89	1.0±0.08	0.0±0.00
26thweek	41.3±1.01 (140)	28.7±0.90	2.6±0.13	0.0±0.00
27th week	42.0±1.01 (140)	36.7±0.58	5.0±0.17	0.5±0.05
28th week	43.3±2.64 (69)	39.7±0.91	7.7±0.18	1.5±0.06
29th week	44.4±2.55 (78)	43.4±0.92	10.6±0.22	2.6±0.08
30th week	44.7±2.73 (69)	45.3±0.88	13.6±0.25	4.1±0.12
31stweek	45.1±2.03 (107)	43.8±0.70	16.6±0.26	5.9±0.16

32nd week	46.0±2.42 (90)	40.1±0.63	19.5±0.27	7.8±0.17
33rd week	46.6±2.24 (100)	39.3±0.61	22.2±0.29	9.8±0.21
34th week	47.6±2.73 (78)	39.0±0.56	24.9±0.31	12.1±0.24
35th week	47.8±2.55 (88)	38.3±0.43	27.5±0.34	14.6±0.26
36th week	48.1±2.57 (88)	37.7±0.50	30.1±0.36	17.0±0.28
37th week	48.5±2.57 (89)	37.4±0.52	32.6±0.39	19.4±0.30
38th week	48.7±2.40 (97)	37.0±0.53	35.2±0.42	21.9±0.32
39th week	49.0±2.65 (86)	35.9±0.32	37.6±0.44	24.2±0.34
40th week	49.0±1.79 (122)	35.8±0.52	40.0±0.45	26.6±0.35
41st week	49.1±1.80 (122)	35.7±0.39	42.4±0.47	28.9±0.37
42nd week	49.2±1.89 (119)	35.0±0.37	44.8±0.48	31.2±0.38
43rd week	49.3±2.40 (99)	34.4±0.44	47.1±0.50	33.4±0.39
44th week	49.4±2.87 (76)	34.1±0.34	49.4±0.51	35.7±0.41
45thweek	50.2±2.76 (84)	33.8±0.53	51.7±0.52	37.9±0.42
46th week	50.9±2.41 (102)	33.6±0.46	54.0±0.54	40.2±0.43
47th week	51.1±1.79 (125)	33.2±0.52	56.2±0.54	42.3±0.44
48th week	51.4±2.28 (108)	32.4±0.43	58.5±0.56	44.5±0.45
49th week	51.4±1.66 (129)	31.7±0.51	60.6±0.57	46.6±0.47
50th week	51.5±1.39 (136)	31.0±0.36	62.7±0.58	48.7±0.48
51st week	51.6±1.95 (120)	30.2±0.47	64.8±0.58	50.7±0.48
52nd week	51.7±1.86 (123)	27.2±0.66	66.6±0.60	52.6±0.49

(n) - Number in parentheses indicate the number of observations
HDEP-Hen day egg production
HHEP- Hen housed egg production
HHHEP- Hen housed hatching egg production

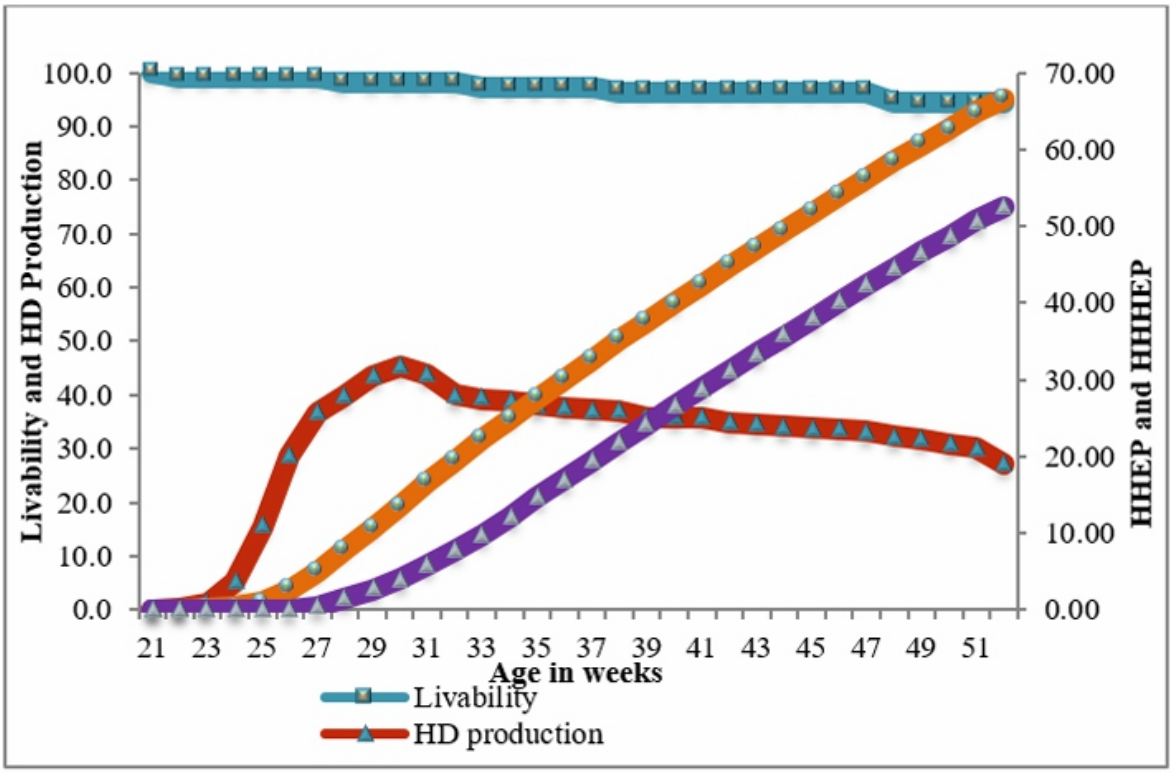

Figure 1. Reproduction performances of Native chicken

4.5. Egg Production

The mean (±S.E.) hen day egg production (HDEP), hen housed egg production (HHEP) and hen housed hatching egg production (HHHEP) of the Native chicken maintained at CPPM, Hosur from 21 to 52 weeks is presented in Table 4. The graphical representation of HD, HHEP and HHHEP of the Native chicken variety from 21 to 52 weeks of age is depicted in Figure 1.

The result revealed that the HDEP reached peak production at 30th week of age with 45.3 per cent. The HDEP between 37 and 45 per cent was maintained between 27th and 38th week of age and HDEP between 30 and 37 cent was maintained between 39th and 51st week of age. The HHEP (No.) of the Native chicken variety at 40th week and 52nd week of age was 40.0±0.45 and 66.6±0.60, respectively. The result indicates that the variety showed good post peak sustainability of egg production. The HHHEP (No.) of the Native chicken variety at 40th week and 52nd week of age was 26.6±0.35 and respectively. The result on HHHEP indicates that variety produced noticeable number of HHHEP suitable for hatching egg production and subsequent chick production.

The observed egg production in the present study is higher than the egg production observed in native chicken of Mysore division of Karnataka [9], in native chicken of Bangalore division of Karnataka [10], in Aseel chicken at 40th week in Hyderabad [18], in native chicken of Belgaum division of Karnataka [5], native chicken in Gulburga division of Karnataka [19]. The egg production however is comparable with egg production of Aseel chicken at Hyderabad [12], Aseel chicken at 40th week of age [14], Aseel chicken at 52nd week [18] and in TANUVAS Aseel chicken [20].

The observation of the study indicates that the variety is comparable with Aseel or other native chicken varieties of India in egg production and hence, the variety could be used under backyard rearing for production of premium price native chicken eggs.

4.6. Fertility and Hatchability

The mean (±S.E.) fertility, hatchability (Total egg set, Hatching egg set) and Chicks per parent of the Native chicken variety maintained at CPPM, Hosur from 21 to 52 weeks is presented in Table 5. The graphical representation of fertility, hatchability and chick per parent of the Native chicken variety from 21 to 52 weeks of age is depicted in Figure 2.

The result revealed excellent fertility (per cent) in Native chicken variety which had ranged between 93.1±0.21 on 52nd week and 95.9±0.32 at 34th and 35th week with an average of 94.68±0.17 per cent. Similarly, the recorded hatchability (TES) in the present study ranged between 82.8±0.55 on 28th week and 88.1±0.07 at 34th week with an average hatchability of 85.70±0.39 per cent on TES, 89.87±0.37 per cent on FES. The study recorded 25.8±1.03 and 45.9±1.44 chicks per dam at 40th and 52nd weeks of age, respectively.

Table 5. Mean (±S.E.) Hatchability performance of Native chicken

Age	Fertility (per cent)	Hatchability (per cent)		Chick per parent
		TES	FES	
26th week	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
27th week	94.0±0.38	79.9±0.56	83.1±0.76	0.7±0.03
28th week	94.1±0.14	82.8±0.55	86.3±0.32	1.5±0.02
29th week	94.5±0.18	83.8±0.09	87.3±0.18	2.6±0.10
30th week	94.7±0.10	84.4±0.47	88.5±0.44	4.0±0.29
31st week	94.9±0.09	86.0±0.34	89.8±0.23	5.6±0.36
32nd week	95.4±0.10	86.6±0.80	90.4±0.56	7.2±0.45
33rd week	95.6±0.26	87.6±0.60	91.3±0.27	9.1±0.55
34th week	95.9±0.32	88.1±0.07	91.6±0.37	11.3±0.61
35th week	95.9±0.32	87.9±0.24	91.4±0.30	13.4±0.68
36th week	95.8±0.33	87.4±0.27	90.8±0.24	15.5±0.74
37th week	95.5±0.33	87.4±0.29	91.1±0.23	17.6±0.82
38th week	95.6±0.33	87.7±0.25	91.0±0.26	19.7±0.89
39th week	95.4±0.46	87.0±0.22	90.8±0.61	21.8±0.94
40th week	95.2±0.50	87.4±0.05	91.1±0.54	23.8±0.99
41st week	95.2±0.73	87.0±0.27	90.9±0.64	25.8±1.03
42nd week	95.1±0.71	86.7±0.17	90.7±0.68	27.8±1.08
43rd week	94.8±0.63	86.4±0.12	90.8±0.69	29.8±1.10
44th week	94.7±0.66	86.3±0.13	90.7±0.87	31.7±1.13
45th week	94.6±0.67	86.3±0.25	90.6±0.70	33.6±1.17
46th week	94.3±0.63	86.1±0.17	90.5±0.69	35.5±1.22
47th week	93.8±0.56	85.2±0.06	90.5±0.61	37.4±1.25
48th week	93.6±0.34	84.4±0.12	89.9±0.34	39.2±1.29
49th week	93.4±0.43	84.7±0.32	89.7±0.41	41.0±1.33
50th week	93.3±0.59	83.8±0.38	89.5±0.51	42.7±1.35
51st week	93.4±0.45	83.8±0.44	89.4±0.52	44.4±1.36
52nd week	93.1±0.21	83.3±0.20	88.9±0.16	45.9±1.44
Average	94.68±0.17	85.70±0.39	89.87±0.37	45.9±1.44

n - Number of observations from four replicates

The observed fertility and hatchability were higher than the observed fertility and hatchability of Aseel chicken in Hyderabad [12], Aseel chicken in Bangladesh [13], Aseel chicken in Hyderabad [17], TANUVAS Aseel chicken [20, 21], Aseel chicken at Faizabad [22] and improved Aseel and Aseel at Hyderabad [23] and comparable with fertility and hatchability of Non-descript chicken in Bangladesh [24] and Kaunayen chicken in Manipur [25].

The observation of the study indicates that the Native chicken variety could be utilized for production of germplasm by the farming community.

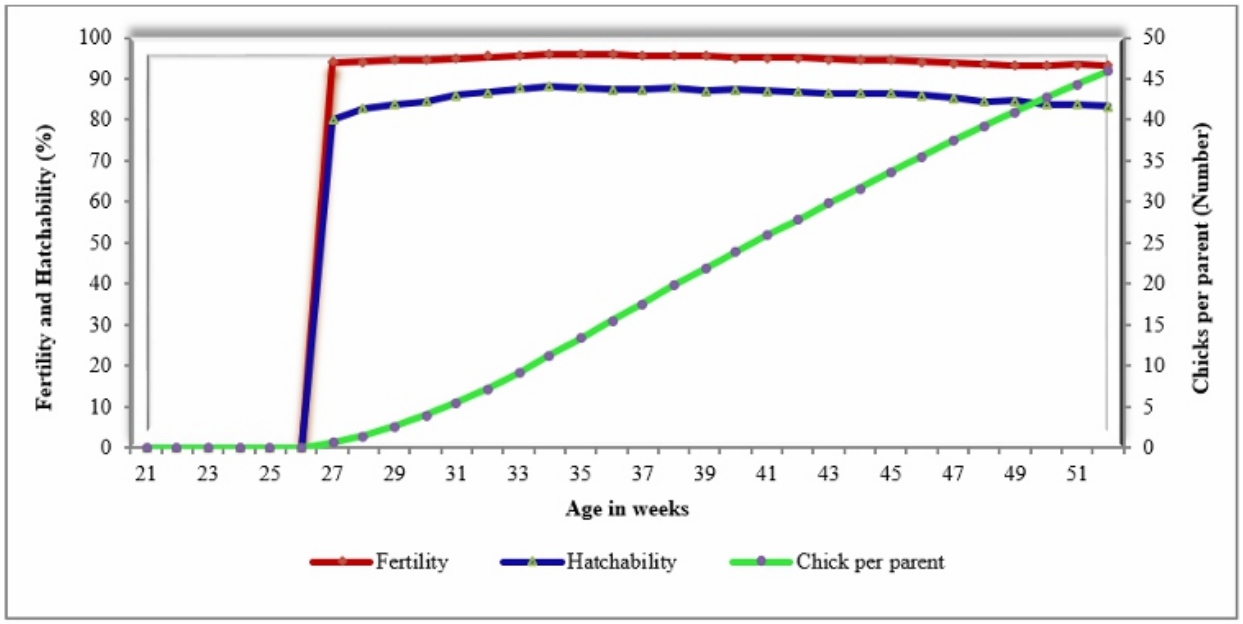

Figure 2. Hatchability performances of the Native chicken variety

5. Summary and Conclusions

The result of reproduction traits revealed that the age at sexual maturity was 149 days of age and the result on reproduction traits revealed hen housed egg production (No.) of 40.0±0.45 and 66.6±0.60 up to 40th and 52nd week, respectively. The result also revealed that the Native chicken variety had fertility per cent ranged between 93.1±0.21 and 95.9±0.32 with an average of 94.68 per cent. Similarly, the recorded hatchability (TES) in the present study ranged between 82.8±0.55 and 88.1±0.07 with an average hatchability of 85.70 percent.

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ISSN: 2331-5083

Impact of Chronic Paraquat Toxicity on Lipid Peroxidation Activity of Indian Major Carp Cirrhinus Mrigala Ham.

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ABSTRACT

Paraquat Dichloride, a broad-spectrum herbicide, used for the killing of aquatic weeds in aquaculture ponds and reservoirs was tested on extensively cultured Indian Major Carp (IMC) Cirrhinus mrigala Ham.. For this purpose, fingerlings of this economically important fish were exposed to the sub-lethal concentrations (LC1/20th and LC1/10th) of paraquat continuously for 30 days by using the static renewal bioassay method. Post Exposure, the lipid peroxidation activity in 4 vital organs of the fish viz. gill, muscle, liver, and brain were estimated by E. D. Wills's method to rectify paraquat's toxicity. Annotated findings specified, a highly significant ($p<0.001$) chronological increase in lipid peroxidation activity (LPO) of gill, muscle, and liver tissues respectively in the LC1/10th concentration group, while a moderately significant ($p<0.01$) increase in LPO activity of brain tissue in the same group. While the gill and the brain tissue of the LC1/20th group showed moderately significant ($p<0.01$) and significant ($p<0.05$) increases in the LPO activity respectively. All changes showed their dependency on the time and concentration factor of the toxicant. Concluding the study, it was stated that, chronic paraquat sub-lethal exposure significantly increased the LPO activity in the vital tissues of Cirrhinus mrigala Ham. thus supporting its highly toxic nature and an immediate need to restrict its use as much as possible.

Keywords Paraquat Dichloride, Chronic Toxicity, Cirrhinus Mrigala Ham., Lipid Peroxidation Activity

1. Introduction

Paraquat Dichloride (C12H14Cl2N2) 75-305-73-0 (CAS) is a quaternary nitrogen bipyridyl weedicide produced commonly in the form of brown color concentrated liquid consisting of 10-30% strong dichloride salt of it, sold the brand name Gramoxone [1] by agrochemical company 'SYNGANTA'. In humans, paraquat ingestion causes hazardous effects in different organ systems. Such paraquat toxicity has no firm existing antidote therapy [2] [4]. The direct spray of paraquat on matured food crops to desiccate them for better marketability reasons is the current most alarming issue that has the highest chances of residual paraquat in the daily diet of humans and animals causing large-scale epidemic issues [5] [6]. Such application of paraquat across various platforms of terrestrial and aquatic ecosystems has led to its far-reaching residues in soil and water that conclusively enter into food chains [7]. Paraquat enters into aquatic ecosystems through surface run-offs, leaching, atmospheric deposition, drifting, etc., and accumulates into various organisms that reside in waters profoundly the fishes [8]. Numerous water bodies viz. Bois d'Orange River, Choc River, Cul-de-Sac River, Roseau Dam, Roseau River, Choc River, Cul-de-Sac River, Soufriere River, Soufriere Dam, Cannelles River, have been found to contain paraquat [9]. The overall health and equilibrium of aquatic creatures may be negatively impacted by the

direct application of paraquat to weeds in ecosystems [10]-[13] thus leading to severe acute as well as chronic toxicity in aquatic organisms. Fish's digestive system, skin, and gills may absorb paraquat from the residual surface waters [11] hence the study of its effects on fish is an important marker for the assessment of its eco-toxicity.

Pesticide poisoning has a key molecular mechanism called Lipid Peroxidation Activity (LPO) [14]. It is a detrimental attack of reactive oxygen species (ROS) that damages tissues and organs by oxidative stress [15]-[17]. Biota exposed to ambient pollutants may significant ROS activity, which may prevent the protective antioxidant system from eliminating them, resulting in oxidative stress and damage [18]-[21]. LPO is crucial for aquatic species since they have a much higher concentration of polyunsaturated fatty acids (PUFA) than terrestrial animals [22]. This study documented the changes in LPO activity as a consequence of malondialdehyde (MDA), a byproduct of PUFA peroxidation that occurs inside cells.

The present study was intended to investigate such toxic effects of paraquat dichloride on the lipid peroxidation activity in the fingerlings of commercially important Indian Major Carp Cirrhinus mrigala Ham., as grassroots biomarkers of pesticide nuisance to the health of aquatic animals useful in the assessment of environmental risks.

2. Materials and Methods

2.1. Procurement and Rearing of Experimental Animal

The Government Fish Seed Production Center, Dhom (Wai), Satara District, Maharashtra State, India, supplied Cirrhinus mrigala Ham. fingerlings (mean weight- 3.18±0.21 gm. and mean length- 4.72±0.44 cm) for this study in large plastic bags sustained by oxygen. Fish were sanitized in a lab setting by being dipped for two minutes in a 0.1% KmNO4 solution. After that, they were placed in well-aerated glass aquariums with regular dechlorinated faucet water, where fish underwent 15 days of acclimatization to room temperature. Standard procedures as illustrated in APHA [23] were used in the experiment to determine the physicochemical parameters of the water. Obtained values were as follows: Temperature 26.2 ºC, pH ranges 7.1-7.6, Dissolved oxygen (DO) content 5.61-6.13 mg/L, liberal CO2 14.27 ± 0.47 mg/L, hardness 119.38 ± 3.72 mg/L, phosphate content 0.5 ± 0.03 mg/L, content 1.11 ± 0.26 mg/L. During acclimatization and experimental procedures, 2 percent fish food (Taiyo Discovery) of the fishes' average body weight was fed to them every day. Natural photoperiod was maintained. During the acclimatization, the aquarium water changed every 24 hours to discard food remnants and fecal matter that can cause unnecessary stress in the enclosed water system. The water quality parameters were checked weekly to ensure normal conditions. Removal of any dead fish was done immediately to avoid possible water quality deterioration. After 15 days of acclimatization, the fish to be used for the experiment were screened critically for indication of physical damage, disease, stress, and mortality. Any suspected fishes were discarded immediately and only the healthy fishes were selected for the study. Before initiation of experimental protocols, the fish were acclimatized to well-aerated 22-liter capacity plastic containers for 7 days, during which they were to be exposed to the toxicant. 24 hours before the test, feeding was discontinued to reduce the effect of vomiting and excess animal excreta due to the toxicant, but later, after 24 hours of exposure, the feeding was restored at 2% of their body weight.

2.2. Exposure to Paraquat

The herbicide paraquat dichloride commercially sold under the brand name Gromoxone (24%w/w) by Syngenta was used as a toxicant in the present study. Before the experiment, a study was conducted to determine the mortality rate of fishes exposed to various concentrations of paraquat by the static renewal bioassay method. The data so obtained was processed by Finney's Probit analysis to obtain the LC50 (105 ppm) value for the toxicant used. Both the sub-lethal concentrations viz. LC1/20th and LC1/10th concentrations used in this study were derived from this LC50 value. For the current experiment, three clear, openmouthed, cylindrical plastic jars with a volume of 22 liters were arranged side by side in a row. To sustain the desired level of dissolved oxygen present in the water, each jar received continuous, appropriate aeration. All containers were filled with 20 liters of clean dechlorinated tap water and 10 fish were released in each of them. The fish in the first jar functioned as a control group because they weren't subjected to any toxicant. The fingerlings in the second and the third jars were subjected to paraquat doses of LC1/20th (pre-calculated: 5.25 ppm) and LC1/10th (pre-calculated: 10.5 ppm) for 30 days each (chronic toxicity). At every 24 hours, the water medium and the toxicant in all the containers were replaced with fresh water and toxicant, to maintain the optimum concentrations throughout the experiment. Any dead fish if observed were removed immediately from the container and buried underground in follow land away from domestic areas. Live juvenile fingerlings from all three jars were euthanized after 30 days of exposure, and the LPO activity in their gills, muscles, and brains was examined in each organ separately. The remains of the euthanized fish too were buried underground in same area where dead fish were buried.

2.3. Analysis of the Lipid Peroxidation Activity (LPO)

In the current investigation, the changed levels of lipid peroxidation in all 4 tissues were estimated using E. D. Wills's protocol [24]. The reaction mixture necessary homogenize the tissues was presumably prepared fresh. To make the reaction mixture, 1 ml of Phosphate Buffer Saline (PBS, pH 7.4), 1 ml of 1 mM FeCl3, 0.01 ml of Chlorotetracycline, and 1 ml of 75 mM Ascorbic Acid (AA) were blended together. Following that, 10 ml of this reaction mixture was used to homogenize 100 mg of fresh tissue. This mix was used as a stock. Following that, 1 ml of this stock solution was divided into three test tubes (triplicates), and 1 ml of distilled water, 1 ml of 20% TCA, and 2 ml of 0.67% thiobarbituric acid (TBA) were all added to each of these triplicates. In an additional test tube, a blank was simultaneously made by mixing 2 ml of distilled water with 1 ml of 20% TCA and 2 Following that, for 15 minutes, all test tubes were submerged in a bath of boiling water. The test tubes were cooled for 15 minutes and centrifuged at 1000 g for 10 minutes. Then, using a spectrophotometer, the absorbance readings of the supernatants so acquired were measured against the blank at 532 λmax. Using 1.56 x 105 M-1 cm-1 as its molar extension coefficient, the lipid peroxidation levels were estimated as nano-moles (nM) of MDA generated per milligram (mg) of tissue (nmol MDA/mg tissue). The following formula was used to determine the level of MDA.

$$\text{MDA / mg tissue} = \frac{\text{O.D. of the sample}}{(0.156)\,(1)}$$

Where,

0.156 = 1 mM Malondialdehyde solution's absorbance at 532 nm in a cell that is 1 cm thick.

1 = Amount of tissue taken in mg, present in 1 ml of a sample.

The final data from all the groups was expressed in Arithmetic Mean (AM) ± Standard Deviation (SD) format. Utilizing the unequal variance (2-sample) (heteroscedastic) approach of "student's T-Test" with "two-tailed distribution", the significance level was determined. If $p < 0.05$ it means it showed a significant change. If $p < 0.01$ it showed a moderately significant change and, if $p<0.001$ it showed highly significant change.

3. Results

The readings representing the effects of paraquat poisoning on the lipid peroxidation activity of Cirrhinus mrigala Ham. gills, brain, muscle tissue, and liver in the control group, LC1/20th concentration set, and LC1/10th concentration group after chronic exposure (30 days) are represented in Table 1. In the control group fish Cirrhinus mrigala Ham., the LPO activity was found to follow the Brain > Liver > Muscle > Gills sequence.

The lipid peroxidation activity in gill tissue showed a moderately significant ($p<0.01$) increase in the LC1/20th group (t.stat = -7.9009) while it showed a highly significant ($p<0.001$) increase in the LC1/10th group (t.stat = -17.901). The lipid peroxidation activity in muscle tissue showed a highly significant ($p<0.001$) increase in the LC1/10th group (t.stat = -16.1532). The lipid peroxidation activity tissue showed a significant ($p<0.05$) increase in the LC1/20th group (t.stat = -3.6360) while in the LC1/10th group (t.stat = -13.026) it showed a highly significant ($p<0.001$) increase. The lipid peroxidation activity in brain tissue showed a significant ($p<0.05$) increase in the LC1/20th group (t.stat = -3.5901) while it showed a moderately significant ($p<0.01$) increase in the LC1/10th group (t.stat = -4.884). The postexperimental lipid peroxidation activity in the four tested tissues was in the order Liver > Brain> Gill > Muscle in the LC1/20th group while in the LC1/10th group it was in the order, Brain > Liver > Gill > Muscle.

Table 1. Effect of Paraquat Dichloride on the Lipid Peroxidation activity in different tissues of the fish *Cirrhinus mrigala Ham.* after chronic exposure

Groups	Lipid peroxidation activity (nM of MDA/mg wet wt. of tissue)			
	Gill	Muscle	Liver	Brain
Control Group	1.76±0.14	1.92±0.14	4.98±0.12	5.12±0.33
$LC_{1/20th}$	3.15±0.26 **	3.03±0.72	6.01±0.47 *	5.9±0.16 *
$LC_{1/10th}$	4.84±0.26 ***	4.6±0.24 ***	6.8±0.2 ***	7.43±0.74 **

(Values in table no 1 are expressed as Arithmetic Mean of (n= 6); ±SD), *= $p<0.05$ (significant), **= $p<0.01$ (moderately significant), ***= $p<0.001$ (highly significant)

The results of the current study illustrate the significance levels of MDA activity that are directly proportional to LPO activity in vital tissues of fish after exposure to paraquat. LC1/10th group show highly significant MDA activity in comparison to control group while LC1/20th group shows a just significant impact on MDA activity as compared to control. Thus the higher concentration (LC1/10th) of paraquat tends to make a highly significant impact on LPO activity in vital tissues of exposed fishes as compared to the lower concentration (LC1/20th) of paraquat.

4. Discussion

Lipid peroxidation results from oxidative injury driven by ROS action that disrupts cell anatomy and physiology [25] [26]. Fish are useful markers of pollutants that allow for early detection of aquatic issues related to environmental health [27] [28]. Internal constituents of fish are harmed when toxicants promote the aberrant creation of ROS and it surpasses the intrinsic defense system of the fish. The term "oxidative refers to this phenomenon [29]. Pesticide impacts causing oxidative stress had been widely investigated as a potential mechanism illustrating their toxicity and degradability, in a variety of tissues [30]. Malondialdehyde (MDA) is a byproduct of LPO. MDA synthesis is a crucial sign of induced oxidative stress driven by free radicals that harm biological membrane constituents [31]. The findings of the current study reveal noticeably higher rates of MDA activity in all four tissues, i.e. the gill, muscle, liver, and brain subjected to Paraquat at both LC1/20th and LC1/10th concentrations. These elevated levels of MDA are a sign of the peroxidation of lipids set on by Paraquat poisoning in key fish tissues. The harmful effects of Paraquat may have resulted in the oxidation and redox-cycling NADPH that potentially releases a large amount of ROS, which ultimately led to oxidative stress and damaged essential tissues via lipid peroxidation. The generated malondialdehyde also reacts actively with other biomolecules like proteins, changing their structures, properties and functions [32]. Lipid peroxidation reduces the nutritional value of edible fish meat, creating health issues as well as the monetary loss for the stakeholders [33]. Persch et al. [34] showed similar results, that Rhamdia quelen fingerlings exposed to multiple paddy herbicides used in integrated rice-fish farming demonstrate a comparable rise in lipid peroxidation rate in their gill, liver, kidney, and muscle tissues. Similarly, [35] studied cadmium induced physiological alterations in Nile tilapia and reported increased lipid peroxidation activity with elevated levels of toxicant as compared to control group.

5. Conclusions

The present study's findings suggest that paraquat poisoning can have a significant detrimental effect on nontargeted creatures like fish because it elevates the levels of lipid peroxidation activity in their vital organs. Increased LPO activity has the potential to impair fish's ability operate optimally and maintain its internal homeostasis, which might result in fish death, growth retardation, and poorer-quality fish meat, which might harm the stakeholders' nutritional needs as well as their health and exacerbate aquatic pollution. LPO activity can also function as a reliable biomarker of how anthropogenic stresses affect unintended organisms.

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ISSN: 2331-5083

Select Phytocompounds from Cocculus hirsutus (L.) W. Theob. Extract Having Anti-Cancer Potential as Identified by LC-MS and in Silico Studies

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ABSTRACT

Cancer is one of the leading causes of mortality worldwide. The metabolites of plant sources have been revealing great promise to treat cancers, too. The anticancer potentials of several medicinal plants are now being studied, although ancient medical systems treated patients of such kind. New directions in cancer research are now possible due to modern developments in biological sciences. In view of various recognised properties of Cocculus, the plant is utilised in the indigenous systems of medicines to treat a wide variety of diseases. Many important secondary metabolites from Cocculus hirsutus (L.) W. Theob. plant have been reported by earlier workers. The previous studies and findings on anticancer activity of crude extract of this plant, encourage to further investigate the Phyto/bio-compounds of extracts with Liquid Chromatography-Mass Spectroscopy (LC-MS) technique and by using in-silico tool to make rational correlations of the properties. By LC-MS technique, phytocompounds were identified from the crude methanolic extract of Cocculus hirsutus. Chemoinformatics tools were employed to screen the phytocompounds and predict the potential anticancer activities of these compounds. Through modern tool in-silico virtual screening software, the potentials of such phytocompounds as drugs and as leads against disease were investigated. The comparative analysis was made between the known activities of the highly used/ approved/standard anticancer plant-derived drugs and predicted activities of the fifteen compounds identified from the extract that were under investigation. In terms of activity, these compounds closely resemble to the approved/standard/recognised plant-derived anticancer drugs. The compounds like the 2,3-Dihydrogossypetin, Trilostane, Nonanoic acid, Irinotecan, Euphornin, Salannin and Gnididilatin are predicted to have drug-likeness. These findings would help reaching the desired target-based medicines for the dreaded disease like cancer. Irinotecan, the semi-synthetic approved/standard Camptothecin anti-cancer alkaloid, agent an (having Topoisomerase I inhibitor activity), is reported for the first time from natural source, like Cocculus hirsutus plant. This plant is in the list of ethnomedicines too and can be considered as a potential source of a drug candidate for the treatment of various types of cancers.

Keywords : Cancer, Camptothecin Derivative, Irinotecan, Antitumor, Anticarcinogenic, Antineoplastic, Chemoprotective, Chemopreventive, Carcinoma, Cocculus Hirsutus

1. Introduction

Cancer is a group of diseases that have claimed the lives of around 10 million people (nearly one in every six fatalities), in 2020 and the disease thus becomes the leading cause of mortality worldwide [1]. Today, many wellknown anticancer substances have been identified and purified from medicinal plants e.g., Taxols/ taxanes (Paclitaxel, Docetaxel) from Taxus spp., Vinca alkaloids from Catharanthus spp.

Vinca from Catharanthus spp. (alkaloids such as Vinblastine, Vincristine and Vindesine from Catharanthus roseus (L.) G.Don), Camptothecin from Camptotheca acuminata Decne., Podophyllotoxins from Podophyllum peltatum L., etc. Many of these compounds are used successfully to treat various types of cancers [2]. The semisynthetic derivatives/analogs of Paclitaxel (Cabazitaxel), Vinblastine (Vinorelbine), Camptothecin (Irinotecan and Topotecan), Podophyllotoxins (Etoposide and Teniposide) are also utilized in the treatment [2]. In the area of cancer research, there has been a great contribution of natural products with reference to the discovery and development of the final drug entity. For instance, of all the approved/standard antitumor/ anticancer drugs worldwide, 79% of drugs are natural/ natural derivatives/ natural inspired, restricting the share of the pure synthetic drugs to 21% only [3].

Vāsanavela plant in Marāṭhī (Pātāla garuḍī in Sanskrit) refers to the accepted species Cocculus hirsutus (L.) W. Theob. [4] Syn. Cocculus hirsutus (L.) Diels Syn. Cocculus villosus DC. Syn. Menispermum hirsutum L. of Family: Menispermaceae, Order: Ranunculales. It is a perennial climber found as a common weed all over Maharashtra state of India. Due to various therapeutic properties of Cocculus, it is found in ancient medical local literature being frequently utilised in the indigenous systems of medicines to treat a wide variety of diseases. When the leaves of this plant are crushed and stirred in water, there is formation of a green semi-solid mucilaginous mass, which makes it a valuable medicine for gonorrhea and other disorders that require demulcents [5,6,7]. This plant is also reported to have antidiarrheal potential in ethnomedicine [8]. Many important secondary metabolites from Cocculus hirsutus plant have been reported by earlier workers. The therapeutic activities of the plant are attributed to various active principles, mainly the isoquinoline alkaloids such as hirsutine [9]; cohirsine [10]; cohirsinine [11]; cohirsitine/cohrisitine [12]; cohirsitinine [13]; haiderine [14]; jamtine-N-oxide [15]; jamtinine [16], and shaheenine [17], which have been isolated from the aerial parts of the plant. The bis-benzyl-isoquinoline alkaloids, like coclaurine, magnoflorine, cocsuline-N-2-oxide, trilobine and isotrilobine have also been found in the plant [18]. The triterpenoids like hirsudiol [19] have been extracted from the plant. In vitro moderate anticancer activity of crude alkaloidal extract of the rhizome of Cocculus hirsutus (L.) Diels was noticed against breast melanoma and renal cancer cell lines [20]. In vitro cytotoxic activity of Cocculus hirsutus whole plant methanolic extract observed against HeLa cell line (Human cervical cancer cell line) [21]. An immortal cell line called HeLa is employed in research. This is the oldest and most widely utilised human cell line [22], named after Henrietta a 31-year-old African-American mother of five, who passed away from cancer on October 4, 1951 [23,24]. The line, which was derived from her cervical cancer cells, was found to be extraordinarily durable and prolific, enabling extensive usage in scientific research [25,26]. The activity was attributed to the bisbenzyl-isoquinoline alkaloids present in the extract [21]. It was further noted that there is significant in vitro and in vivo antitumor activity of Cocculus hirsutus against breast cancer cell lines in [27]. When in vitro cytotoxic activity of methanolic extract of the leaves of Cocculus hirsutus was investigated on human breast cancer cell lines, the findings revealed a dose-dependent reduction in cell viability and suppression of cell growth [28]. The docking studies were carried out by in silico techniques to show that the phytoconstituents of Cocculus such as coclaurine, haiderine, and lirioresinol can potentially bind with the select targets of hepatocellular carcinoma [29]. These studies and findings encourage further investigation of the phyto/ biocompounds of the extracts of Cocculus hirsutus with LC-MS technique and use of in-silico tool to make rational correlations, which would help reaching the future desired target-based medicines for the dreaded diseases like cancer.

2. Materials and Methods

Collection and Identification of Plant Material The wild twigs of Cocculus hirsutus (L.) W. Theob. (Figure 1) were collected from the tribal areas of Ahmednagar district, Maharashtra state, India. These were identified with the help of floras and the herbarium specimen (Figure 2) was authenticated from the BSI, Pune [Authentication Number: SNRJ-4 Cocculus hirsutus (L.) Theob. (Menispermaceae) dated 23.12.2020].

Figure 1. Cocculus hirsutus (L.) W. Theob. climber

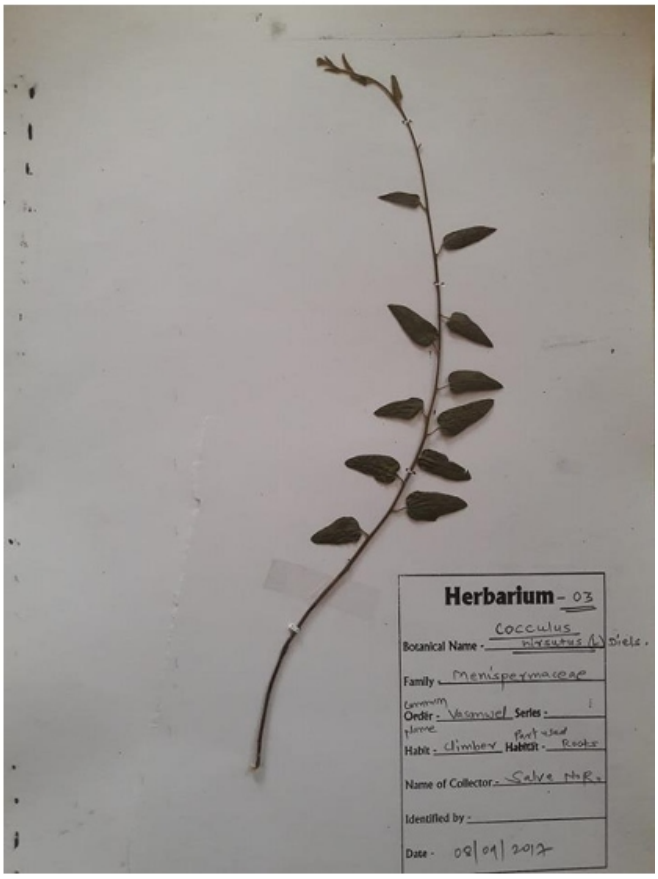


Figure 2. Herbarium Cocculus hirsutus (L.) W. Theob

Preparation of Powder and Extract

The whole plant (except roots) materials were cleaned, shade dried, powdered and extracted with methanol for 810 hours using Soxhlet apparatus. The extract was then filtered through Whatman No.1 filter paper and evaporated to get the concentrated semi-solid mass. Extraction process was repeated to get sufficient extract. The methanolic extracts were used for LC-MS analysis.

LC-MS Analysis

The LC-MS [LC model (Agilent make) and MS model (Q-TOF LC/MS) make] instrument was used to find the presence of the proper phytocompounds in the methanol extract of the plant parts. The experiment was conducted at the Venture Center (Entrepreneurship Development Center), NCL, Pune's Center for Applications of Mass Spectrometry (CAMS) facility. Databases from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and (https://www.ebi.ac.uk/chebi/), were utilised to identify the phytochemical compounds. These databases were used to gather the SMILES (The simplified molecular-input lineentry system) for the compounds. Through the use of analysis software, these SMILES were employed for further compound analysis.

PASS Analysis

To assess the overall biological potentials of the 15 compounds identified from the plant extract, the program "Way2Drug PASS (Prediction of Activity Spectra for Substances) online" (http://www.way2drug.com/passonline/) was employed. The Pa, which stands for a compound's "probability active" in an extract and the Pi for "probability to be inactive" for a particular biological activity were considered for each compound. All compounds with are predicted to be active, and the probability that a real experiment will confirm that activity is high if Pa>0.7 [30]. The results of PASS prediction of these 15 compounds from the extract are presented in table 1. The biological activity spectra of some well-known anticancer plantderived drugs were also predicted using the PASS software. The results of predictions are presented in table 4. The comparative analysis was made between the known activities well-known/approved/standard anticancer plant-derived drugs and the predicted activities of the compounds from the extract of Cocculus hirsutus that was under investigation.

Cytotoxicity for Tumour Cell Lines

For in silico prediction of cytotoxicity potential (for tumour cell lines) of the 15 compounds identified from the plant extract, the freely available web-service "CLC-Pred (Cell Cytotoxicity Predictor)" (http://www.way2drug.com/Cellline/) was employed [31]. The results of this prediction presented in table 2.

Drug-likeness and Lead-likeness

A free online tool called (http://www.swissadme.ch) was used to assess how close the compounds were in activity to drug-likeness and leadlikeness. SMILES strings, which aid in assessing compounds' potential for drug-likeness and lead-likeness after taking ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties account, were uploaded to the site for this purpose. The

results of this assessment are presented in table 3.

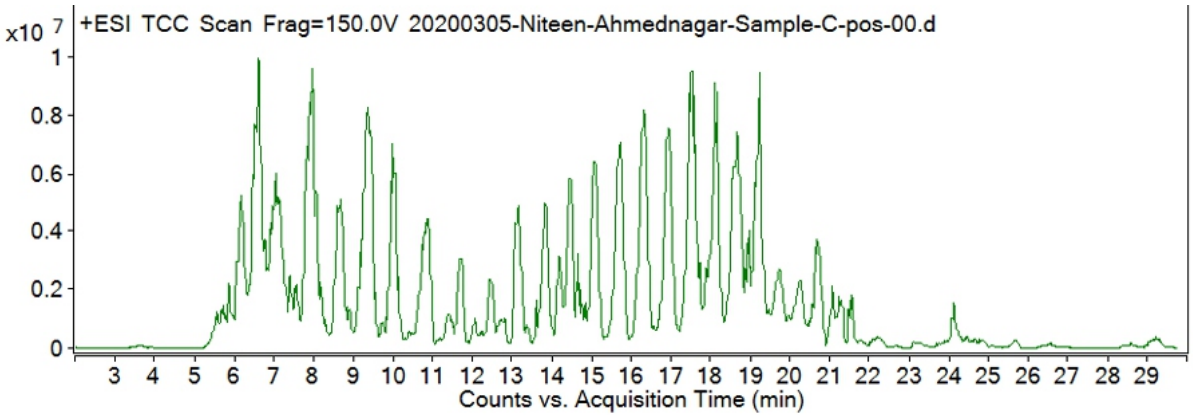
Molecular Docking and in vitro/ in vivo Studies Review

This is neither the scope nor the intention of this paper to carry out the molecular docking analysis of the individual phytocompounds of the extract or to carry out the in vitro/ in vivo studies for anticancer activities. But the previous work carried out on the docking analysis and in vitro/ in vivo investigations for some of the phytocompounds (as also found after investigation in the present work) are reviewed in brief in the discussion part to correlate to the in silico results conducted in the present study.

3. Results and Discussion

Results

The results are presented in the following Graph 1, Tables 1-5 and Figures 3-4.



Graph 1. LC- MS chromatogram of methanolic extract of *Cocculus hirsutus* (L.) W. Theob. aerial parts

Table 1. Prediction of Biological Activity of Compounds from *Cocculus hirsutus* (L.) W. Theob. Extract by Chemo-informatics Approach

Sr. No.	RT	Mass	Name of the compound with Formula	Biological/ Drug Activity [d*]	PASS Prediction for Desired Activities	
					Pa	Pi
1.	6.07	594.157	Saponarin – $C_{27}H_{30}O_{15}$	Chemopreventive	0,942	0,002
				TP53 expression enhancer	0,935	0,004
				Anticarcinogenic	0,911	0,002
				Cytostatic	0,866	0,005
				Antineoplastic	0,833	0,008
				HIF1A expression inhibitor	0,826	0,010
				Radioprotector	0,813	0,004
2.	7.02	450.115	2',3,4,4',6'-Peptahydroxychalcone 4'-O-glucoside – $C_{21}H_{22}O_{11}$	Caspase 3 stimulant	0,969	0,002
				Chemopreventive	0,939	0,002
				Anticarcinogenic	0,898	0,003
				Antineoplastic	0,821	0,009
				TP53 expression enhancer	0,780	0,013
				Antileukemic	0,703	0,005

3.	7.13	320.052	2,3-Dihydrogossypetin – $C_{15}H_{12}O_8$	TP53 expression enhancer	0,967	0,003
				HIF1A expression inhibitor	0,951	0,003
				Anticarcinogenic	0,808	0,005
				Chemoprotective	0,767	0,002
				Chemopreventive	0,728	0,005
				Cytostatic	0,726	0,009
				Antineoplastic	0,715	0,024
4.	7.88	329.198	Trilostane – $C_{20}H_{27}NO_3$	Antineoplastic	0,785	0,014
5.	10.75	158.130	Nonanoic acid – $C_9H_{18}O_2$	Preneoplastic conditions treatment	0,821	0,003
				Centromere associated protein inhibitor	0,747	0,005
				TP53 expression enhancer	0,740	0,019
6.	13.11	322.249	17alpha-Methyl-5alpha-androstane-3beta,11beta,17beta-triol – $C_{20}H_{34}O_3$	Caspase 3 stimulant	0,781	0,007
				Antineoplastic	0,720	0,023
7.	13.83	294.218	13-OxoODE – $C_{18}H_{30}O_3$	Preneoplastic conditions treatment	0,819	0,003
				TP53 expression enhancer	0,739	0,019
				Radioprotector	0,723	0,009
				HIF1A expression inhibitor	0,702	0,020
8.	18.12	586.279	Irinotecan – $C_{33}H_{38}N_4O_6$	Antineoplastic	0,864	0,006
				HIF1A expression inhibitor	0,811	0,011
				Antineoplastic alkaloid	0,790	0,002
				Antineoplastic (solid tumours)	0,749	0,004
				Topoisomerase I inhibitor	0,743	0,001
				Antineoplastic (colorectal cancer)	0,718	0,005
				Antineoplastic (colon cancer)	0,713	0,005
				Antineoplastic (small cell lung cancer)	0,701	0,003

Table 1 continued

9.	18.15	470.413	Hopane-29-acetate – $C_{32}H_{54}O_2$	Antineoplastic	0,826	0,009
				Antineoplastic (lung cancer)	0,751	0,005
10.	19.31	584.299	Euphornin – $C_{33}H_{44}O_9$	Antineoplastic	0,856	0,006
11.	19.72	596.298	Salannin – $C_{34}H_{44}O_9$	Antineoplastic	0,931	0,005
				Antineoplastic (colorectal cancer)	0,850	0,004
				Antineoplastic (colon cancer)	0,848	0,004
				Prostate cancer treatment	0,813	0,004
				Antineoplastic (breast cancer)	0,752	0,005
				Antineoplastic (lung cancer)	0,705	0,006
12.	20.70	584.423	Flavoxanthin – $C_{40}H_{56}O_3$	Antineoplastic	0,949	0,004
				Antileukemic	0,807	0,004
				Chemopreventive	0,785	0,004

13.	20.71	568.427	Lutein – $C_{40}H_{56}O_2$	Antineoplastic	0,913	0,005
				Radioprotector	0,895	0,002
				Chemopreventive	0,877	0,003
				Prostate cancer treatment	0,834	0,003
				TP53 expression enhancer	0,731	0,020
14.	20.94	936.58	1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-3-(Galactosyl-alpha-1-6-Galactosyl-beta-1)-glycerol – $C_{51}H_{84}O_{15}$	Anticarcinogenic	0,899	0,002
				Radioprotector	0,856	0,003
				Chemopreventive	0,850	0,003
				Radiosensitizer	0,746	0,004
15.	21.22	652.324	Gnididilatin (Gnidilatin) – $C_{37}H_{48}O_{10}$	Antineoplastic alkaloid	0,913	0,001
				Antineoplastic	0,889	0,005
				DNA synthesis inhibitor	0,744	0,005
				Antineoplastic (lung cancer)	0,729	0,005
				Protein kinase C alpha inhibitor	0,711	0,002

Table 2. Prediction of cytotoxicity for tumour cell lines of compounds from Cocculus hirsutus (L.) W. Theob. Extract by Chemo-informatics Approac http://www.way2drug.com/Cell-line/

Sr. No.	**Name of the compound with Formula**	**Cell-line with Cell line full name (organism- *Homo sapiens*)**	**PASS Prediction for Desired Activities**		**Tissue and tumor type**
			Pa	**Pi**	
1.	Saponarin – $C_{27}H_{30}O_{15}$	HL-60 (Promyeloblast leukaemia)	0.566	0.020	Haematopoietic and lymphoid tissue Leukaemia
		NCI-H838 (Non-small cell lung cancer. 3 stage)	0.525	0.038	Lung Carcinoma
2.	2',3,4,4',6'-Peptahydroxychalcone 4'-O-glucoside – $C_{21}H_{22}O_{11}$	NCI-H838 (Non-small cell lung cancer. 3 stage)	0.528	0.036	Lung Carcinoma
3.	2,3-Dihydrogossypetin – $C_{15}H_{12}O_8$	NCI-H187 (Small cell lung carcinoma)	0.520	0.011	Lung Carcinoma
		Hs 683 (Oligodendroglioma)	0.523	0.049	Brain Glioma
4.	Trilostane – $C_{20}H_{27}NO_3$	H9 (T-lymphoid)	0.784	0.002	Haematopoietic and lymphoid tissue Leukaemia
5.	Nonanoic acid – $C_9H_{18}O_2$	DMS-114 (Lung carcinoma)	0.558	0.017	Lung Carcinoma
		SK-MEL-1 (Metastatic melanoma)	0.545	0.017	Skin Melanoma
		A2058 (Melanoma)	0.521	0.009	Skin Melanoma
		NCI-H838 (Non-small cell lung cancer. 3 stage)	0.537	0.033	Lung Carcinoma
6.	17alpha-Methyl-5alpha-androstane-3beta,11beta,17beta-triol – $C_{20}H_{34}O_3$	SF-539 (Glioblastoma)	0.701	0.005	Brain Glioblastoma
		UACC-62 (Melanoma)	0.658	0.005	Skin Melanoma
		OVCAR-3	0.605	0.011	Ovarium

		SN12C (Renal carcinoma)	0.582	0.008	Kidney Carcinoma
7.	13-OxoODE – $C_{18}H_{30}O_3$	IGROV-1 (Ovarian adenocarcinoma)	0.616	0.009	Ovarium Adenocarcinoma
		A2058 (Melanoma)	0.504	0.012	Skin Melanoma
		NCI-H838 (Non-small cell lung cancer. 3 stage)	0.510	0.044	Lung Carcinoma

Table 2 continued

8.	Irinotecan – $C_{33}H_{38}N_4O_6$	PC-6 (Small cell lung carcinoma)	0.845	0.004	Lung Carcinoma
		LS174T (Colon adenocarcinoma)	0.767	0.002	Colon Adenocarcinoma
		A549 (Lung carcinoma)	0.709	0.021	Lung Carcinoma
9.	Hopane-29-acetate – $C_{32}H_{54}O_2$	SK-MEL-1 (Metastatic melanoma)	0.556	0.013	Skin Melanoma
10.	Euphornin – $C_{33}H_{44}O_9$	NCI-H838 (Non-small cell lung cancer. 3 stage)	0.635	0.015	Lung Carcinoma
		SK-MEL-1 (Metastatic melanoma)	0.565	0.010	Skin Melanoma
11.	Salannin – $C_{34}H_{44}O_9$	OVCAR-5 (Ovarian adenocarcinoma)	0.735	0.007	Ovarium Adenocarcinoma
		HL-60 (Promyeloblast leukaemia)	0.673	0.010	Haematopoietic and lymphoid tissue Leukaemia
		NCI-H838 (Non-small cell lung cancer. 3 stage)	0.522	0.039	Lung Carcinoma
12.	Flavoxanthin – $C_{40}H_{56}O_3$	PC-3 (Prostate carcinoma)	0.714	0.008	Prostate Carcinoma
13.	Lutein – $C_{40}H_{56}O_2$	PC-3 (Prostate carcinoma)	0.880	0.004	Prostate Carcinoma
		CWR22R (Prostate carcinoma epithelial cell line)	0.837	0.002	Prostate Carcinoma
		LNCaP (Prostate carcinoma)	0.636	0.003	Prostate Carcinoma
14.	1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-3-(Galactosyl-alpha-1-6-Galactosyl-beta-1)-glycerol – $C_{51}H_{84}O_{15}$	BT-549 (Breast ductal carcinoma)	0.654	0.008	Breast Carcinoma
		NCI-H838 (Non-small cell lung cancer. 3 stage)	0.630	0.016	Lung Carcinoma
		SK-MEL-1 (Metastatic melanoma)	0.541	0.018	Skin Melanoma
15.	Gnididilatin – $C_{37}H_{48}O_{10}$	HT-1080 (Fibrosarcoma)	0.894	0.002	Soft tissue Sarcoma
		MCF7 (Breast carcinoma)	0.542	0.043	Breast Carcinoma

Table 3. Prediction of Drug-likeness and Lead-likeness of important compounds from *Cocculus hirsutus* (L.) W. Theob. Extract

Sr. No.	Name of the compound (with Drug type/category)	Druglikeness (Lipinski*)	Leadlikeness
1.	Saponarin – a glucoside	**No;** 3 violations: MW>500, NorO>10, NHorOH>5	**No;** 1 violation: MW>350
2.	2',3,4,4',6'-Peptahydroxychalcone 4'-O-glucoside – a β-D-glucoside	**No;** 2 violations: NorO>10, NHorOH>5	**No;** 1 violation: MW>350
3.	2,3-Dihydrogossypetin – a dihydroflavonols	Yes; 1 violation: NHorOH>5	**Yes**
4.	Trilostane – An epoxy steroid	**Yes;** 0 violation	**Yes**
5.	Nonanoic acid – a saturated fatty acid	**Yes;** 0 violation	**No;** 1 violation: MW<250
6.	17alpha-Methyl-5alpha-androstane-3beta,11beta,17beta-triol – a 3-hydroxy steroid	**Yes;** 0 violation	**Yes**
7.	13-OxoODE – an oxo fatty acid	**Yes;** 0 violation	**No;** 2 violations: Rotors>7, XLOGP3>3.5
8.	Irinotecan – a pyranoindolizino-quinoline	**Yes;** 1 violation: MW>500	**No;** 2 violations: MW>350, XLOGP3>3.5
9.	Hopane-29-acetate – a pentacyclic triterpenoid	**Yes;** 1 violation: MLOGP>4.15	**No;** 2 violations: MW>350, XLOGP3>3.5
10.	Euphornin – a diterpene	**Yes;** 1 violation: MW>500	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
11.	Salannin – a monoterpene	**Yes;** 1 violation: MW>500	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
12.	Flavoxanthin – a carotenoid	**No;** 2 violations: MW>500, MLOGP>4.15	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
13.	Lutein – a carotenoid	**No;** 2 violations: MW>500, MLOGP>4.15	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
14.	1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-3-(Galactosyl-alpha-1-6-Galactosyl-beta-1)-glycerol – a glycosylglycerol derivative	**No;** 3 violations: MW>500, NorO>10, NHorOH>5	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
15.	Gnididilatin – a diterpenoid	**Yes;** 1 violation: MW>500	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5

Table 4. Prediction of Biological Activity of Plant-derived approved/standard Compounds by Chemo-informatics Approach

Sr. No.	Mass	Name of the compound with Formula	Biological/ Drug Activity [d*]	PASS Prediction for Desired Activities	
				Pa	Pi
1.	853.9	Paclitaxel (Taxol) – $C_{47}H_{51}NO_{14}$	Antineoplastic	0,990	0,003
			Antineoplastic (colorectal cancer)	0,976	0,003
			Antineoplastic (colon cancer)	0,975	0,003
			TP53 expression enhancer	0,962	0,003
			Antineoplastic (breast cancer)	0,949	0,003
			Cytostatic	0,948	0,002
			Antineoplastic (ovarian cancer)	0,921	0,003
			Tubulin antagonist	0,920	0,003
			Antimitotic	0,917	0,002
			CYP19A1 expression inhibitor	0,889	0,001
			Microtubule formation inhibitor	0,872	0,000
			Anticarcinogenic	0,866	0,003
			Antineoplastic (lung cancer)	0,862	0,003
			Radiosensitizer	0,833	0,002
			Antineoplastic (lymphoma)	0,797	0,003
			Cancer associated disorders treatment	0,772	0,002
			Antineoplastic (carcinoma)	0,771	0,003
2.	811.0	Vinblastine – $C_{46}H_{58}N_4O_9$	TP53 expression enhancer	0,989	0,002
			Tubulin antagonist	0,975	0,002
			Cytostatic	0,938	0,002
			Antineoplastic alkaloid	0,931	0,001
			Antineoplastic	0,872	0,005
			Beta tubulin antagonist	0,850	0,002
			Antineoplastic (cervical cancer)	0,809	0,003
			Anticarcinogenic	0,809	0,003
			Antimitotic	0,707	0,004
3.	825.0	Vincristine – $C_{46}H_{56}N_4O_{10}$	Cytostatic	0,988	0,001
			Tubulin antagonist	0,978	0,002
			Antineoplastic alkaloid	0,953	0,000
			Antineoplastic	0,884	0,005
			Beta tubulin antagonist	0,791	0,003
			Antineoplastic (cervical cancer)	0,760	0,003
4.	753.9	Vindesine – $C_{43}H_{55}N_5O_7$	Tubulin antagonist	0,965	0,002
			Antineoplastic alkaloid	0,940	0,001
			Beta tubulin antagonist	0,934	0,002
			Cytostatic	0,926	0,003
			TP53 expression enhancer	0,899	0,005
			Antineoplastic	0,834	0,008
			Antineoplastic (cervical cancer)	0,758	0,003

Table 4 continued

5.	348.4	Camptothecin – $C_{20}H_{16}N_2O_4$	Topoisomerase I inhibitor	0,942	0,001
			HIF1A expression inhibitor	0,936	0,004
			Antineoplastic	0,929	0,005
			Antineoplastic (colorectal cancer)	0,839	0,004
			Antineoplastic (solid tumours)	0,835	0,003
			Antineoplastic (colon cancer)	0,833	0,004
			Antineoplastic (small cell lung cancer)	0,796	0,003
			Caspase 3 stimulant	0,772	0,007
			TP53 expression enhancer	0,778	0,013
			Antineoplastic (lung cancer)	0,768	0,005
			Antineoplastic alkaloid	0,731	0,002
6.	414.4	Podophyllotoxin – $C_{22}H_{22}O_8$	Caspase 3 stimulant	0,966	0,002
			Antineoplastic	0,940	0,004
			TP53 expression enhancer	0,867	0,007
			Antineoplastic (lung cancer)	0,849	0,004
			Antineoplastic (small cell lung cancer)	0,847	0,003
			Cytostatic	0,830	0,006
			Antineoplastic (colorectal cancer)	0,772	0,005
			Antineoplastic (colon cancer)	0,771	0,005
			Topoisomerase II inhibitor	0,741	0,003
			Tubulin antagonist	0,718	0,004

Table 5. Prediction of Drug-likeness and Lead-likeness of Plant-derived approved/standard Compounds

Sr. No.	**Name of the compound (with Drug type/category)**	**Druglikeness (Lipinski*)**	**Leadlikeness**
1.	Paclitaxel (Taxol) – an antineoplastic agent	**No;** 2 violations: MW>500, NorO>10	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
2.	Vinblastine – an alkaloid	**No;** 2 violations: MW>500, NorO>10	**No;** 3 violations: MW>350, Rotors>7, XLOGP3>3.5
3.	Vincristine – an alkaloid	**No;** 2 violations: MW>500, NorO>10	**No;** 2 violations: MW>350, Rotors>7
4.	Vindesine – an alkaloid	**No;** 2 violations: MW>500, NorO>10	**No;** 1 violation: MW>350
5.	Camptothecin – an alkaloid	**Yes;** 0 violation	**Yes**
6.	Podophyllotoxin – a lignan	**Yes;** 0 violation	**No;** 1 violation: MW>350

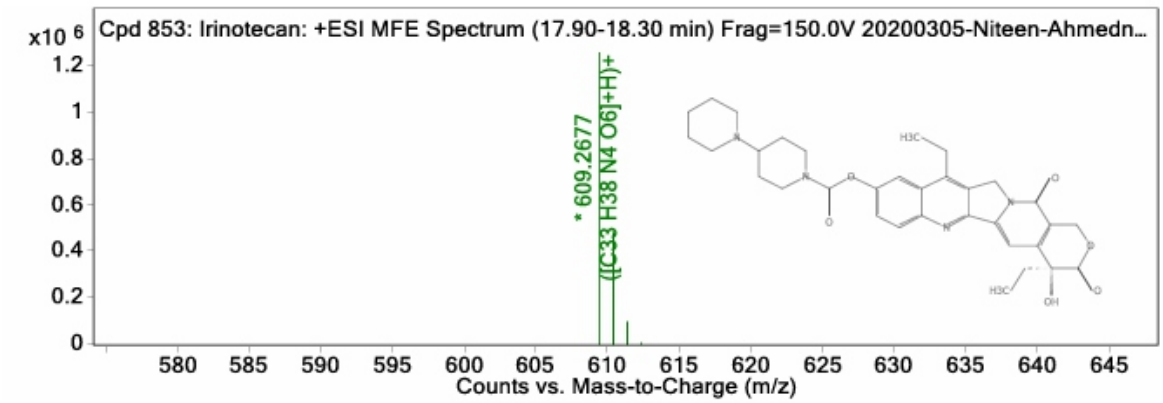


Figure 3. MFE MS Spectrum of Irinotecan

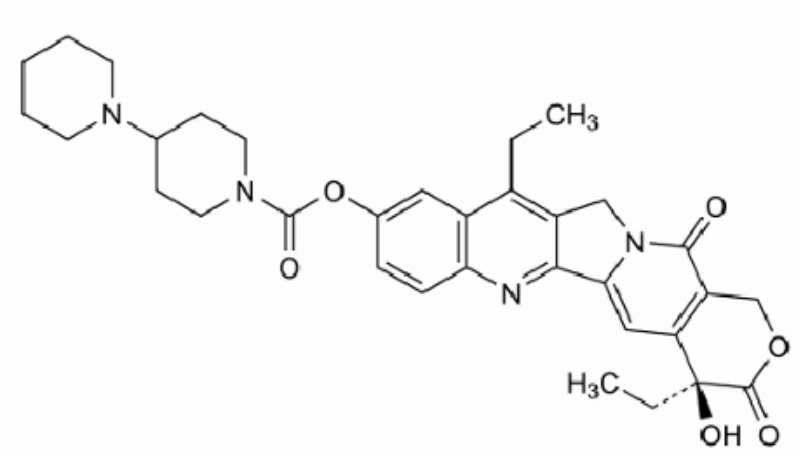


Figure 4. ***Chemical Structure of Irinotecan*** Courtesy: Parviz Norouzi et al (2009) Determination of Anti Colon Cancer Drug, Irinotecan by Fast Fourier Transforms Continuous Cyclic Voltammetry - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Chemical-Structure-of-irinotecan_fig1_228672699 [accessed 13 Jul, 2023]

Discussion

The anti-cancer compounds exhibit diverse mechanisms of actions. Some of those compounds are briefly discussed below with reference to the in silico predicted activities of the standard compounds as well as with the compounds extracted from Cocculus hirsutus (L.) W. Theob plant.

The expression of TP53, a tumor suppressor gene, results in formation of tumor protein 53 (p53) that acts as a tumor suppressor. Protein 53 (p53) controls DNA repair and cell division. If the damage or mutation in DNA is irreparable, the protein stops the cell from dividing and signals apoptosis, preventing the tumor formation [32]. The compounds of the studied plant extract numbered 1, 2, 3, 5, 7 and 13 (Table 1) are predicted to act like TP53 (Tumour protein 53) expression enhancers. The approved/standard plant-derived drugs like Paclitaxel (Taxol), Vinblastine, Vindesine, Camptothecin and Podophyllotoxin (Table 4) are also predicted here by chemo-informatics approach to have TP53 expression enhancer activity.

important transcription factor involved in the development of cancer and thus is used as a target in cancer therapy. It acts differently in oxygenated and non-oxygenated environments. Therefore, by

focusing on HIF1, cancer formation and progression can be controlled. HIF1A inhibitors work at the transcription as well as translation level regulation of HIF1A gene expression [33]. In this study, Camptothecin (Table 4) revealed HIF1A expression inhibitor activity.

Interestingly, the extract compounds numbered 1, 3, 7 and 8 (Table 1) also have been predicted to carry this activity. The process of programmed cell death (apoptosis) is significantly influenced by cysteine proteases (Caspases). Cytotoxic drugs cause apoptosis in the tumour cells, causing the death of tumour cells. Many such substances, both natural and synthetic, have been reported which possess caspase-3-mediated anticancer action through the apoptotic pathway [34]. Camptothecin and Podophyllotoxin (Table 4) are reported Caspase 3 stimulant activity, while the extract compounds numbered 2 and 6 (Table 1) are also predicted to express the similar activity.

Radioprotectors are substances that shield normal cells from the damaging effects of radiation without affecting the radio-sensitivity of malignant cells [35]. The plant extract compounds numbered 1, 7, 13 and 14 (Table 1) are predicted to have radioprotector activity too. Radiosensitizers are substances that target cancer cells selectively and increase the sensitivity of such cells to ionizing radiation reducing the effects of radiation on healthy non-cancerous cells [36]. The extract compound numbered 14 (Table 1) is found to express this activity like that of the Paclitaxel (Taxol) (Table 4).

Isozyme like PKC alpha (Protein Kinase C alpha), is an intracellular signaling protein which plays a significant role in the development of malignant tumors. Cancer treatments make use of PKC alpha inhibitors that interfere with various signaling pathways of tumor cells resulting in the arrested growth and apoptosis [37]. The extract compound Gnididilatin is revealed to have such PKC alpha inhibitor activity. Gnididilatin has been found in Gnidia spp. (Thymelaeaceae) and recorded to have antileukemic activity [38].

Camptothecin (CPT) and its semisynthetic derivatives viz., Irinotecan and Topotecan are TOP I (DNA Topoisomerase I) inhibitors, while Podophyllotoxins and its semisynthetic derivatives viz., Etoposide and Teniposide are TOP II (DNA Topoisomerase II) inhibitors. Topoisomerase I inhibitors interrupt DNA replication in cancer cells leading to the death of those cells [39]. The Topoisomerase II inhibitors interrupt the processes like DNA replication, chromosomal condensation and segregation in tumour cells inducing their apoptosis [40]. DNA Topoisomerase I enzyme makes a single-strand break in DNA backbone while DNA Topoisomerase II makes a double-strand break in DNA backbone. In the present extract, compound Irinotecan was identified (Table 1, compound numbered 8; Figures 3 and 4) and was predicted to possess Topoisomerase I inhibitor activity. Camptothecin is a prototype Topoisomerase I inhibitor. Irinotecan and SN-38 (7-ethyl-10hydroxycamptothecin), both Topoisomerase I inhibitors, are derivatives/analogues of Camptothecin and Irinotecan respectively. Irinotecan is developed from Camptothecin to enhance its solubility for intravenous administration. Irinotecan is a water-soluble prodrug which gets enzymatically converted to active metabolite SN-38 by carboxylesterases in liver. SN-38 is more active and less toxic than Irinotecan in human body [41]. Irinotecan is currently the most popular Camptothecin, with an annual turnover in regulated countries over $1 billion US Irinotecan is the first-line treatment for stomach and colon cancers, especially when combined with fluorouracil and vascular disrupting drugs [42].

Molecular Docking; Earlier Investigations

Irinotecan has been shown to bind more firmly to the therapeutic target proteins MDM2 and Bcl-x, suppressing the activities and promoting the anticancer effects [43]. Irinotecan is also shown to target the receptors such as the protein kinase B, VEGFR-2 (Vascular Endothelial Growth Factor Receptor-2), and procaspase 7 by binding stably to these receptors [44]. Trilostane was shown to inhibit human 3β-HSD1 (3β-Hydroxysteroid Dehydrogenase type 1), a major target enzyme for the treatment of breast cancer, with high affinity [45]. Lutein has been shown to bind more firmly to the HER-2 (Human Epidermal Receptor-2) proteins, suppressing the activities and promoting the antibreast cancer effects [46]. Lutein's ability to inhibit breast cancer cell proliferation is quantitatively comparable to that of the standard chemotherapeutic drugs such as taxanes (paclitaxel and docetaxel) [47]. Salannin was identified as one of the strong inhibitors of the cdk protein in docking studies used to screen anticancer drugs from Azadirachta indica using Saccharomyces cerevisiae as the model system [48].

In vitro/ In vivo- Earlier Investigations

It has been demonstrated through in-vitro HeLa cell line model that Euphornin inhibits the growth of human cervical cancer HeLa cells by inducing programmed cell death and arrest of G2/M cell cycle [49]. Euphornin was also shown to have cytotoxic effects on the mice lung cancer cells (adenocarcinoma LA795) [50]. Gnidilatin exhibited moderate inhibitory activity at the dose of about 80 µg/kg of body weight against the P-388 leukaemia in mice [51]. It has been shown that the particularly inhibits the proliferation of the human cancer cell lines MDA-MB-468 and MCF7 [52]. Trilostane is shown to inhibit the growth of HCC (Hepatocellular carcinoma) cell lines in preclinical trials by inhibiting the activity of the enzyme HSD3B1 (3-hydroxysteroid dehydrogenase type 1). And the US FDA-approved drug sorafenib is found to be more effective when used with trilostane [53]. In all in vitro tested colon and pancreatic cancer cell lines, irinotecan demonstrated concentration- and time-dependent mortal effects. It also showed concentration-dependent prevention of colony formation after being exposed to fresh tumors for 30 minutes [54].

The trials with above described phytocompounds and synthetic compounds are gaining momentum in cancer research due to the natural healing properties of these compounds for which the ancient medical practitioners used those plants expectedly having such compounds. The attempts by the researchers to identify new and more specific bio-compounds, which could have anticancer properties at various cellular levels need clinical satisfaction justifying reverse pharmacology and the rationality of ancient wisdom in present day cancer treatments.

4. Conclusions

The metabolites from plants have great promise for treating deadly diseases like cancer. The anticancer potentials of several medicinal plants are now being studied. New directions in cancer research are now possible due to recent computational developments in biological sciences. It is feasible to start experimental research with substances that look the most promising in in silico predictions. The biological activities of the compounds found in the extract are noted to be virtually identical to those of the established plant-derived anticancer agents. The compounds like 2,3Dihydrogossypetin, Trilostane, Nonanoic acid, Irinotecan, Hopane-29-acetate, Euphornin, Salannin and Gnididilatin are predicted to have drug-likeness. The compounds found in the extract are also found to have nearly identical cytotoxic potential (for tumour cell lines) to that of the established plant-derived anticancer drugs. Irinotecan, the semi-synthetic Camptothecin approved/standard anti-cancer alkaloid, agent an (having

Topoisomerase I inhibitor activity), is reported for the first time from natural source, like Cocculus hirsutus Thus, the plant, Cocculus hirsutus (L.) W. Theob., can be considered as a potential source of a drug candidate for treatment of various types of cancers and should form the part of the screening program of anticancer agents. To develop novel herbal medicine with safe and effective usage in addressing global health challenges related to cancer, further in-depth research, scientific exploration, and pre-clinical and clinical trials are necessary on this plant.

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ISSN: 2331-5083

Toxic Effect of Green Seaweeds on the Larval Instars of Vector Mosquitoes

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ABSTRACT

Seaweed species have been reported for their toxic effects on mosquito larvae. In the present study, the ether, chloroform, acetone and methanol extracts of two green seaweeds, Caulerpa racemosa and Ulva fasciata were tested for toxicity against the second and third instar of Aedes aegypti and Culex quinquefasciatus as per the guidelines of World Health Organization at concentrations of 100, 200, 300, 400 and 500 mg/L for 24 hours. Caulerpa racemosa extracts recorded 100% mortality at the highest concentration on the second and third instar of Aedes aegypti and Culex quinquefasciatus, and maximum larvicidal activity was exhibited by the chloroform extract, and their respective LC50 values were 140.49 and 144.554 mg/L, and 153.704 and 158.313 mg/L. In the case of Ulva fasciata, the chloroform extract exhibited 100% mortality at the highest concentration on the second and third instar of Aedes aegypti and Culex quinquefasciatus, and also the maximum larvicidal activity with LC50 values of 158.358 and 166.025 mg/L; and 154.156 and 187.435 mg/L against the second and third instar larvae, respectively. Overall results indicated that amongst the two green seaweeds tested, Caulerpa racemosa exhibited more activity when compared to Ulva fasciata, and with reference to solvent extracts, the chloroform extract exhibited maximum activity against the larval instars of the vector mosquito species tested. With regard to the vector mosquito species tested, Aedes aegypti was more susceptible than Culex quinquefasciatus, and in the case of instars, second instar larvae were more susceptible than the third instar. In conclusion, the bioassay result of the present study indicated the larvicidal property of the chloroform extract of both the green seaweeds against the larval instars of vector mosquitoes, which encourages further investigation on its bioactive compounds that might own virtuous larvicidal properties when isolated in pure form.

Keywords Green Seaweeds, Caulerpa racemosa, Ulva fasciata, Solvent Extracts, Larvicidal Activity, Aedes aegypti, Culex quinquefasciatus

1. Introduction

Mosquitoes are ravaging humans and other animals for generations. The mosquito vector-borne diseases, malaria, dengue, chikungunya, filariasis, and Japanese encephalitis comprehend the global disease incidence as the control of these disease transmitting vectors are challengeable globally [1]. The synthetic/chemical immense aerial, usage terrestrial of and many aquatic insecticides offers logistic problems on the environment and causes resurgence of different mosquito-borne diseases, and has stimulated investigations for environmentally safe, bio-degradable and target specific insecticides against mosquitoes [2]. This situation has focused more attention on discovering novel beneficial natural products, and has immensely contributed to stimulating the increasing interest in unconventional and unexplored sources of natural products. In this context, seaweeds have attracted

much attention over the past four decades [3]. Marine macroalgae popularly known as seaweeds are groups of ecologically important vegetation of oceanic ecosystem that contain secondary metabolites [4], with economically potential renewable and extraordinary sustainable resources [5]. Researchers have found that the seaweeds possess good mosquitocidal properties [6], like bio-insecticides derived from that of terrestrial plants [7-9]. The idea of using marine macroalgae to combat mosquito larvae is not new [10,11]. Certain species of green macroalgae kill larvae primarily because they are indigestible, while blue-green algae offer possibilities for delivery as larvicides since they act as neuro and hepatotoxins to mosquito larvae [12,13]. The long history of seaweed based products in insecticide research on discovering new active agents in seaweeds in growing, and on top of that, many reports have revealed seaweeds' profound insecticidal properties on mosquitoes [6]. Considering the biodiversity of seaweeds in tropical regions, there is a need to study their larvicidal potential, since active metabolites of seaweeds possess larvicidal properties [6,14]. Therefore in the present study, the crude extracts of green seaweeds, viz., Caulerpa racemosa and Ulva fasciata were tested for their toxicity on the larval instars of Aedes aegypti and Culex quinquefasciatus, the principal vectors for dengue and filarial fever, respectively.

2. Materials and Methods

2.1. Seaweed Collection

Green seaweed species, viz., Caulerpa racemosa (Forsskål) J.Agardh (Caulerpaceae) commonly called sea grapes and Ulva fasciata Delile (Ulvaceae) popularly known as sea lettuce were collected by hand picking the intertidal zone of Rameswaram, Tamil Nadu, India (8 46 N, 78 9 E and 9 14 N, 79 14 E), rinsed in water to remove sand and other particles, and transferred to laboratory in sterilized ziplock bags for further studies. Taxonomical identification and confirmation of the collected seaweeds was done at the Marine Algal Research Station Mandapam, Tamil Nadu, India with the help of morphological key characters and identification manual [15-17].

2.2. Preparation of Seaweed Extracts

The two green seaweeds were shade dried at room temperature for a week, and were powdered with the aid of a mixer grinder. The powdered sample of each seaweed species (250g) was sequentially suspended in a selective solvent system ranging from non-polar to polar (petroleum ether, chloroform, acetone and methanol) for 72 hours, (750 mL for each solvent), and then soxhlated for eight hours to obtain crude extracts [18]. Thereafter, each extracted sample was filtered using Whatman No.1 paper, and the filtered sample was individually centrifuged at 5000 rpm for 10 minutes at 4°C, and the supernatant was collected in a separate flask. Each extract was then concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland). The final concentrated crude solvent extract of each seaweed obtained was individually stored in sterile air tight bottles and kept in a refrigerator until further use. Prior to this, the percentage of yield of extraction of the crude extracts was calculated.

2.3. Test Vector Mosquitoes

The eggs of Aedes aegypti and egg rafts of Culex quinquefasciatus were procured from Centre for Research in Medical Entomology (CRME), Indian Council of Medical Research (ICMR), Madurai, Tamil Nadu, India. Larvae of each test vector mosquito species were reared separately in larval enamel

trays containing dechlorinated water, and were fed with a finely powdered mixture of biscuits and dry yeast in a ratio 3:1.

2.4. Larvicidal Bioassay

According to the guidelines of the World Health Organization [19] with minor modifications, bioassays were performed on healthy F1 generation of laboratory larvae of Aedes aegypti and Culex quinquefasciatus. Serial dilution of 1.0% stock solution of each crude solvent seaweed extract yielded requisite test concentrations (100, 200, 300, 400, and 500 amount of test solution. Bioassays in triplicates with an overall of three trials were performed on the early second and third instar of the two vector mosquitoes numbering twenty each added separately into glass beakers (250mL) holding distilled water and test concentration for each replicate apiece trial. In parallel, control tests were performed with distilled water (250mL) as a positive control, and Tween 80 (1.0mL) dissolved in distilled water served as a negative control. Larvae were fed with larval feed during the experiment. Larval mortality was observed 24 hours after treatment and larvae were scored dead when they displayed no signs of movement when probed by needle at their respiratory siphon. The activity level of seaweed extracts based on the average percent larval mortality were construed as moderately active (50–75%), weakly active (25–50%), and inactive (<25%) [20].

2.5. Data Analyses

Larval mortality in percentage was calculated, and Abbott's formula [21] was applied when larval mortality of control ranged between 5% and 20%. Statistical analysis was run in IBM SPSS version 27 statistics software [22]. Statistical analysis of all mortality data of larvicidal activity was subjected to probit, chi-square and regression analysis. One-way analysis of variance and Duncan's multiple comparison significant difference post hoc tests were to significantly determine whether mortality in treated bioassays differed from controls and at which concentration in particular; and also whether significant differences in response between solvents of the extract group existed. The differences were considered as significant at $P \leq 0.05$ level with significance set at 95% confidence.

3. Results

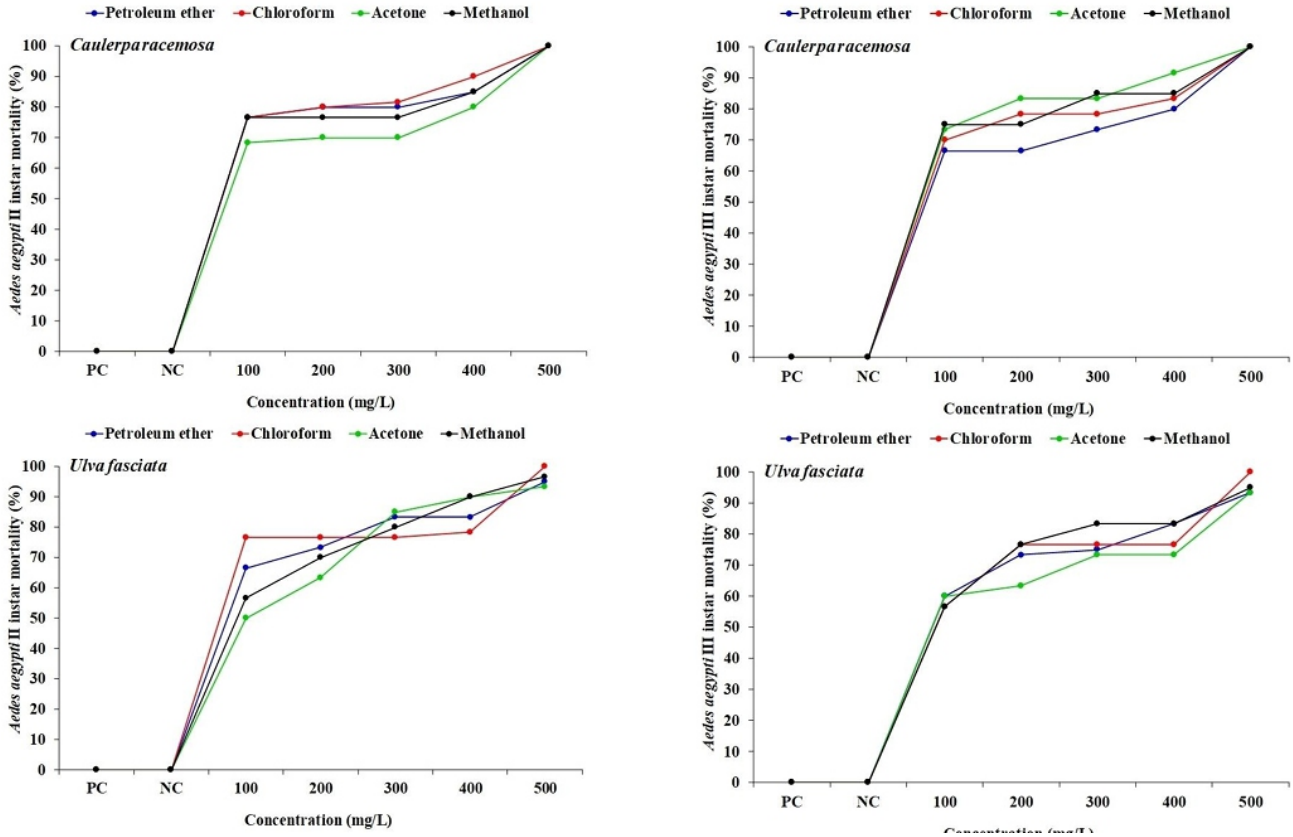
The percentage yield of solvent (petroleum ether, chloroform, acetone and methanol) extracts of Caulerpa racemosa was 0.98, 3.32, 0.98 and 1.98, and for Ulva fasciata it was 1.98, 2.48, 3.19 and 2.93 respectively. Caulerpa racemosa extracts recorded 100% mortality at the highest concentration on the second and third instar of Aedes aegypti. Caulerpa racemosa petroleum chloroform and methanol extracts were highly active as they exhibited >75% larvicidal activity against the second instar of Aedes aegypti at the lowest concentration of 100 mg/L, and against the third instar it was the methanol extract which exhibited 75% larvicidal activity (Table 1; Figure 1). Maximum larvicidal activity was exhibited by the chloroform extract at 500 mg/L against the second and third instars of Aedes aegypti, and their respective Lc50 values were 140.409 and 144.554 mg/L (Table 2). In the case of Ulva fasciata, its chloroform extract exhibited 100% mortality at the highest concentration on the second and third instar of Aedes aegypti, and was highly active against the second instar of Aedes aegypti at the lowest concentration (Table 1; Figure 1). The maximum larvicidal activity was exhibited again by the chloroform extract at 500 mg/L, and the LC50 value was 158.358 and 166.025 mg/L, against the second and third instar larvae of Aedes aegypti, respectively (Table 2). Against the larval instars of Culex

quinquefasciatus, all extracts of Caulerpa racemosa and the chloroform extract of Ulva fasciata displayed 100% mortality at 500 mg/L (Table 3; Figure 1). However, one of the extracts of both the green seaweeds was highly active against the larval instars of Culex quinquefasciatus at the lowest concentration. Maximum larvicidal activity was again exhibited by the chloroform extract green seaweed species, and their LC50 values were and 158.313 mg/L; and 154.156 and 187.435 mg/L against the second and third instars of Aedes aegypti and Culex quinquefasciatus respectively (Table 4).

Table 1. Toxicity of green seaweed extracts on the larval instars of *Aedes aegypti*

Concentration (mg/L)	II instar				III instar			
	Petroleum ether	Chloroform	Acetone	Methanol	Petroleum ether	Chloroform	Acetone	Methanol
				Caulerpa racemosa				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	15.33 ± 2.51^{b1}	15.33 ± 2.30^{b1}	13.66 ± 0.57^{b1}	15.33 ± 0.57^{b1}	13.33 ± 2.08^{b1}	14.0 ± 2.0^{b1}	14.66 ± 0.57^{b1}	15.0 ± 1.0^{b1}
200	16.0 ± 2.64^{bc1}	16.0 ± 2.0^{bc1}	14.0 ± 2.0^{b1}	15.33 ± 0.57^{b1}	13.33 ± 2.08^{b1}	15.66 ± 2.08^{b12}	16.66 ± 0.57^{bc2}	15.0 ± 1.0^{b12}
300	16.0 ± 2.64^{bc1}	16.33 ± 1.52^{bc1}	14.0 ± 3.0^{b1}	15.33 ± 3.78^{b1}	14.66 ± 2.08^{b1}	15.66 ± 3.05^{b1}	16.66 ± 3.05^{bc1}	17.0 ± 1.0^{c1}
400	17.0 ± 1.0^{bc1}	18.0 ± 1.0^{cd1}	16.0 ± 2.64^{bc1}	17.0 ± 1.0^{bc1}	16.0 ± 1.0^{b1}	16.66 ± 2.08^{b1}	18.33 ± 1.15^{c1}	17.0 ± 1.73^{c1}
500	20.0 ± 0.0^{c}	20.0 ± 0.0^{d}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{c}	20.0 ± 0.0^{d}
				Ulva fasciata				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	13.33 ± 2.88^{b12}	15.33 ± 3.05^{b2}	10.0 ± 2.0^{b1}	11.33 ± 1.15^{b12}	12.0 ± 1.0^{b1}	11.33 ± 2.08^{b1}	12.0 ± 1.0^{b1}	11.33 ± 1.15^{b1}
200	14.66 ± 2.08^{b1}	15.33 ± 2.51^{b1}	12.66 ± 1.52^{c1}	14.0 ± 1.73^{c1}	14.66 ± 2.08^{bc1}	15.33 ± 2.30^{b1}	12.66 ± 1.15^{bc1}	15.33 ± 2.30^{c1}
300	16.66 ± 2.08^{bc1}	15.33 ± 2.51^{b1}	17.0 ± 1.73^{d1}	16.0 ± 2.64^{cd1}	15.0 ± 2.64^{c1}	15.33 ± 3.21^{b1}	14.66 ± 2.08^{c1}	16.66 ± 1.52^{c1}
400	16.66 ± 2.51^{bc1}	15.66 ± 3.78^{b1}	18.0 ± 1.0^{de1}	18.0 ± 2.0^{de1}	16.66 ± 2.08^{c1}	15.33 ± 4.04^{b1}	14.66 ± 2.08^{c1}	16.66 ± 2.08^{c1}
500	19.0 ± 0.0^{c1}	20.0 ± 0.0^{e2}	18.66 ± 0.57^{e1}	19.33 ± 0.57^{e12}	18.66 ± 0.57^{d1}	20.0 ± 0.0^{e2}	18.66 ± 0.57^{d1}	19.0 ± 1.0^{d12}

PC: Positive control; NC: Negative control; Data are mean±standard deviation of larval mortality of three replicates of three trials; Different numerical superscripts in column indicate values significant than respective PC and NC, and different superscript alphabets in rows indicate values significant between the extracts at $p\leq0.05$ level by one way ANOVA followed by Duncan's multiple comparison post hoc test performed; Similarity in alphabetical and numerical superscripts in rows and columns indicate no significant variation



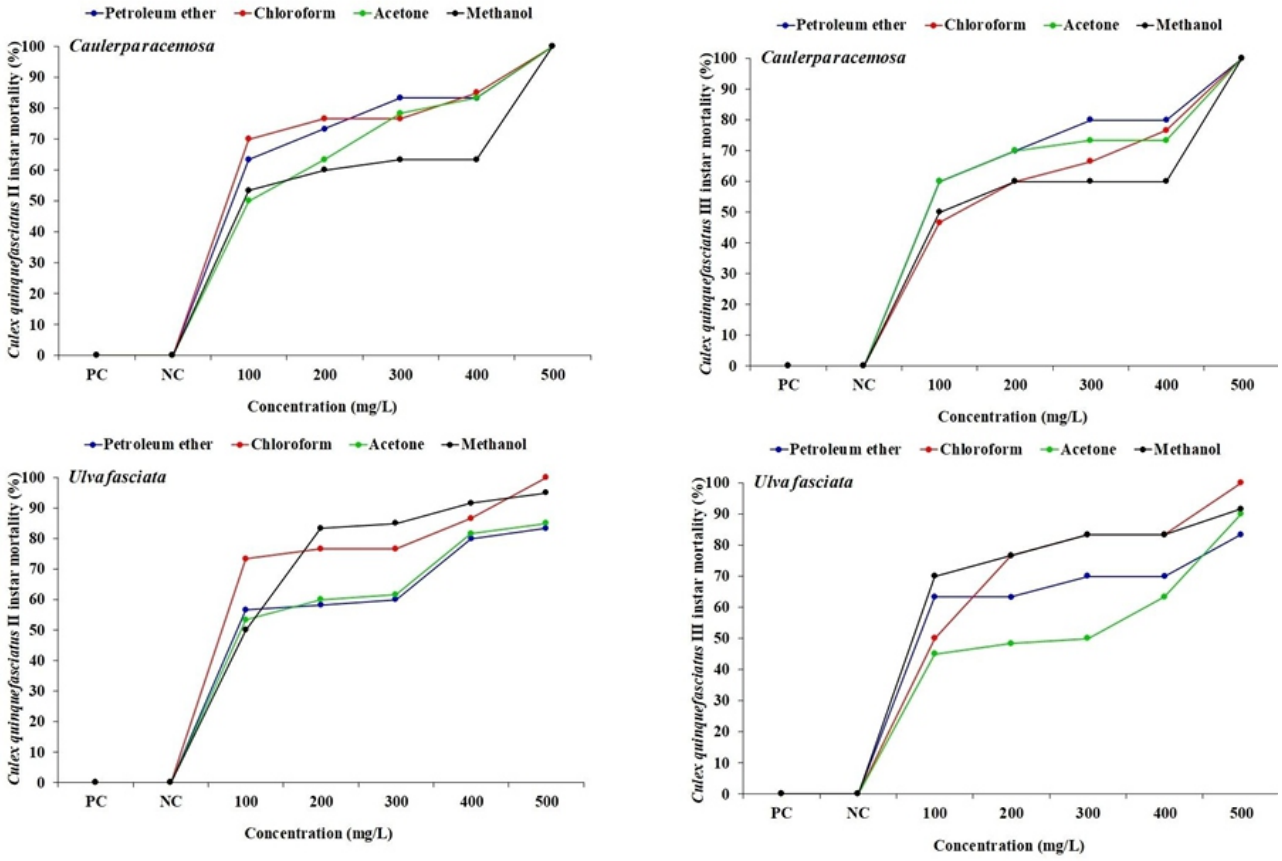


Figure 1. Percent larval mortality of vector mosquitoes on exposure to green seaweed extracts

Table 2. Probit analysis and associated statistical inferences of green seaweed extracts against the larval instars of *Aedes aegypti*

Seaweed extracts	LC_{50} (mg/L)	95% CL (LB-UB)	LC_{90} (mg/L)	95% CL (LB-UB)	Regression equation	R^2	χ^2	P value
II instar								
				Caulerpa racemosa				
Petroleum ether	151.412	83.182-211.038	361.664	288.743-501.453	Y=-0.511+0.112x	0.921	25.890*	0.002*
Chloroform	140.409	85.581-190.989	311.369	250.794-422.510	Y=-35.95+0.152x	0.847	15.875*	0.007*
Acetone	157.082	95.381-213.709	347.764	279.405-477.747	Y=-1.666+0.123x	0.960	24.465*	0.004*
Methanol	153.078	88.242-210.726	357.562	287.122-489.609	Y=-0.411+0.182x	0.949	5.727*	0.767†
				Ulva fasciata				
Petroleum ether	158.425	100.308-212.633	343.430	277.887-464.877	Y=5.633+0.125x	0.987	14.789*	0.001*
Chloroform	158.358	78.039-228.600	367.978	285.557-548.996	Y=8.607+0.222x	0.914	26.470*	0.002*
Acetone	173.850	140.613-207.225	331.918	288.620-396.332	Y=11.42+0.215x	0.915	28.888*	0.001*
Methanol	167.337	125.143-208.963	333.448	281.216-418.732	Y=2.914+0.212x	0.980	52.073*	0.001*
III instar								
				Caulerpa racemosa				
Petroleum ether	184.924	126.719-239.923	413.990	341.498-544.078	Y=-1.366+0.054x	0.930	13.987*	0.123†
Chloroform	144.554	78.085-203.041	338.672	269.158-470.597	Y=-13.53+0.240x	0.960	48.247*	0.001*
Acetone	183.294	118.774-243.480	416.856	338.782-565.168	Y=-0.622+0.088x	0.973	12.087*	0.208†
Methanol	155.575	89.864-214.140	363.192	291.152-500.808	Y=-2.25+0.039x	0.964	11.848*	0.222†
				Ulva fasciata				
Petroleum ether	173.185	122.432-222.015	362.227	300.747-468.599	Y=0.311+0.096x	0.896	21.004*	0.013†
Chloroform	166.025	116.529-213.512	343.472	284.899-443.783	Y=3.522+0.212x	0.967	44.377*	0.001*
Acetone	193.219	138.468-246.815	406.108	336.590-530.362	Y=0.966+0.202x	0.948	25.673*	0.002*
Methanol	178.565	114.652-239.069	378.008	304.827-520.081	Y=0.911+0.170x	0.975	38.319*	0.001*

LC_{50} & LC_{90}: Lethal concentration that kills 50% and 90% of the treated larvae respectively; CL: Confidence limits; LB: Lower bound; UB: Upper bound;

χ^2: Chi-square value; R^2: Coefficient of determination; *Values significant at p≤0.05 level; †Values not significant at p≤0.05 level

Table 3. Toxicity of green seaweed extracts on the larval instars of *Culex quinquefasciatus*

Concentration (mg/L)	II instar				III instar			
	Petroleum ether	Chloroform	Acetone	Methanol	Petroleum ether	Chloroform	Acetone	Methanol
				Caulerpa racemosa				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	12.66 ± 1.52^{b1}	14.0 ± 1.73^{b1}	10.0 ± 1.0^{b1}	10.66 ± 4.61^{b1}	12.0 ± 1.0^{b1}	9.33 ± 1.52^{b1}	12.0 ± 1.0^{b1}	10.0 ± 4.35^{b1}
200	14.66 ± 2.08^{bc1}	15.33 ± 1.52^{bc1}	12.66 ± 1.52^{c1}	12.0 ± 5.19^{b1}	14.0 ± 1.73^{bc1}	12.0 ± 1.0^{bc1}	14.0 ± 1.73^{b1}	12.0 ± 4.35^{b1}
300	16.66 ± 1.52^{c1}	15.33 ± 2.30^{bc1}	15.66 ± 0.57^{d1}	12.66 ± 5.50^{b1}	16.0 ± 2.64^{cd1}	13.33 ± 2.08^{cd1}	14.66 ± 2.08^{b1}	12.0 ± 5.19^{b1}
400	16.66 ± 1.52^{c2}	17.0 ± 2.0^{c2}	16.66 ± 1.52^{d2}	12.66 ± 1.51^{b1}	16.0 ± 2.64^{cd1}	15.33 ± 2.51^{de1}	14.66 ± 3.51^{b1}	12.0 ± 1.0^{b1}
500	20.0 ± 0.0^{d}	20.0 ± 0.0^{d}	20.0 ± 0.0^{e}	20.0 ± 0.0^{c}	20.0 ± 0.0^{d1}	20.0 ± 0.0^{e1}	20.0 ± 0.0^{c1}	20.0 ± 0.0^{c1}
				Ulva fasciata				
PC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
NC	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}	0.0 ± 0.0^{a0}
100	11.33 ± 1.15^{b1}	14.66 ± 2.08^{b2}	10.66 ± 0.57^{b1}	10.0 ± 0.0^{b1}	12.66 ± 1.52^{b23}	10.0 ± 1.0^{b12}	9.0 ± 2.64^{b1}	14.0 ± 1.73^{b3}
200	11.66 ± 2.08^{b1}	15.33 ± 2.30^{b12}	12.0 ± 1.0^{b1}	16.66 ± 2.88^{c2}	12.66 ± 3.05^{b12}	15.33 ± 2.30^{c2}	9.66 ± 3.21^{b1}	15.33 ± 2.30^{b2}
300	12.0 ± 2.0^{b1}	15.33 ± 3.21^{b1}	12.33 ± 2.51^{b}	17.0 ± 2.64^{c1}	14.0 ± 1.73^{b12}	16.66 ± 2.88^{cd2}	10.0 ± 2.0^{b1}	16.66 ± 2.88^{bc2}
400	16.0 ± 2.64^{c1}	17.33 ± 2.51^{bc1}	16.33 ± 2.51^{c1}	18.33 ± 1.52^{cd1}	14.0 ± 4.35^{b1}	16.66 ± 3.05^{cd1}	12.66 ± 4.16^{b1}	16.66 ± 3.05^{bc1}
500	16.66 ± 1.52^{d1}	20.0 ± 0.0^{c3}	17.0 ± 1.73^{d12}	19.0 ± 0.0^{d23}	17.66 ± 2.51^{c1}	20.0 ± 0.0^{d1}	18.0 ± 1.73^{c1}	18.33 ± 1.15^{c1}

PC: Positive control; NC: Negative control; Data are mean±standard deviation of larval mortality of three replicates of three trials; Different numerical superscripts in column indicate values significant than respective PC and NC, and different superscript alphabets in rows indicate values significant between the extracts at $p\leq0.05$ level by one way ANOVA followed by Duncan's multiple comparison post hoc test performed; Similarity in alphabetical and numerical superscripts in rows and columns indicate no significant variation.

Table 4. Probit analysis and associated statistical inferences of green seaweed extracts against the larval instars of *Culex quinquefasciatus*

Seaweed extract	LC_{50} (mg/L)	95% CL (LB-UB)	LC_{90} (mg/L)	95% CL (LB-UB)	Regression equation	R^2	χ^2	P value
II instar								
				Caulerpa racemosa				
Petroleum ether	180.214	107.808-245.259	426.392	342.941-589.574	Y=-0.877+0.226x	0.910	37.572*	0.001*
Chloroform	153.704	90.580-211.606	340.151	271.166-474.764	Y=-4.313+0.223x	0.946	51.561*	0.001*
Acetone	205.526	153.473-256.434	437.323	368.520-553.190	Y=-13.82+0.202x	0.896	39.344*	0.001*
Methanol	228.341	158.541-301.716	478.571	384.568-677.606	Y=-0.926+0.219x	0.977	67.713*	0.001*
				Ulva fasciata				
Petroleum ether	196.483	128.727-262.523	422.020	339.282-590.159	Y=6.477+0.104x	0.944	16.142*	0.064†
Chloroform	154.156	88.775-213.930	339.277	269.094-478.124	Y=49.75+0.116x	0.914	18.825*	0.002*
Acetone	204.031	158.026-250.652	404.875	343.239-507.564	Y=27.14+0.174x	0.834	26.183*	0.002*
Methanol	155.492	112.760-197.677	303.597	252.979-388.354	Y=11.25+0.194x	0.968	30.425*	0.001*
III instar								
				Caulerpa racemosa				
Petroleum ether	187.748	120.109-250.698	422.870	342.451-577.616	Y=-0.857+0.161x	0.962	86.788*	0.001*
Chloroform	158.313	100.748-211.798	347.138	281.665-467.661	Y=-0.222+0.223x	0.940	94.129*	0.001*
Acetone	223.440	176.480-271.308	455.934	389.068-565.686	Y=-0.188+0.229x	0.891	64.408*	0.001*
Methanol	242.981	174.597-317.775	508.522	409.953-716.744	Y=-5.821+0.179x	0.898	62.415*	0.001*
				Ulva fasciata				
Petroleum ether	205.762	155.654-256.624	414.111	347.267-530.269	Y=0.88+0.104x	0.970	17.494*	0.042*
Chloroform	187.435	126.354-246.117	400.118	326.195-539.914	Y=0.577+0.220x	0.929	38.516*	0.001*
Acetone	257.543	202.033-320.065	498.686	414.948-655.618	Y=0.511+0.215x	0.931	25.234*	0.003*
Methanol	171.893	120.361-221.873	345.222	284.928-451.554	Y=0.088+0.182x	0.990	54.257*	0.001*

LC_{50} & LC_{90}: Lethal concentration that kills 50% and 90% of the treated larvae respectively; CL: Confidence limits; LB: Lower bound; UB: Upper bound; χ^2: Chi-square value; R^2: Coefficient of determination; *Values significant at $p\leq0.05$ level; †Values not significant at $p\leq0.05$ level

4. Discussion

A broad spectrum of seaweed species have been reported for their toxic effects on mosquito larvae [6,23], and in the present study the crude solvent extracts of Caulerpa racemosa and Ulva fasciata were reported for larvicidal action on Aedes aegypti and Culex quinquefasciatus. Overall results indicate that amongst the two green seaweeds tested, Caulerpa racemosa exhibited more activity when compared to Ulva fasciata, and with reference to solvent extracts, the chloroform extract exhibited maximum activity

when compared to Ulva fasciata, and with reference to solvent extracts, the chloroform extract exhibited maximum activity against the larval instars of the vector mosquito species tested. With regard to the vector mosquito species tested, Aedes aegypti was more susceptible than Culex quinquefasciatus, and among the instars, second instar larvae were more susceptible than the third instar.

Earlier studies of Caulerpa racemosa with different solvents tested against mosquito species have been reported. Its petroleum ether-acetone extracts exhibited LC50 values <100 mg/L against Aedes aegypti and Culex quinquefasciatus larvae [24]; ethanol extract showed toxicity against the fourth instar larvae of Aedes aegypti, Culex quinquefasciatus, Anopheles stephensi due to the presence of phytoconstituents like terpenoids, fatty acids, saponins, steroids, alkaloids, tannins, glycosides,
carbohydrates, flavonoids, proteins and a compound caulerpin, and their respective LC50 values were 0.055, 0.067 and 0.066 µg/mL [25]; methanol extract showed effective activity against Culex tritaeniorhynchus as it ruptured the midgut of larvae [26], and reported LC50 values of >1000 µg/mL against Aedes aegypti and Aedes albopictus [23]; hexane, chloroform, ethyl acetate, acetone and methanol extracts showed LC50 values of 910.2, 728.4, 579.9, 811.8, 886.0 ppm against Aedes aegypti and their activity was due to presence of terpenoids, tannins and phenolics [27]. The results of the present study provided far better results based on LC50 values when compared with the above mentioned previous studies. Besides these, Caulerpa racemosa exhibited profound larvicidal activity with better LC50 values than other species of Caulerpa, wherein Caulerpa scalpelliformis acetone extract reported LC50 value of 53.70 mg/L against Aedes aegypti [28], which is an exception when compared to the present study, and LC50 value of 338.91 ppm against Culex pipiens, larvae and caused >70% larval mortality at 24 hours [29]. Its ethanol extract showed LC50 value of 0.07, 0.06 and 0.06 µg/mL against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi [25], and ethanol extracts of Caulerpa chemnitzia, Caulerpa scalpelliformis and Caulerpa taxifolia against Aedes aegypti with LC50 values of 2500, 2000 and 1900 ppm respectively [30].

In the case of Ulva fasciata too, results of the present study provided pronounced larvicidal effects with Lc50 values <200 mg/L, which was better when equated with earlier studies with different solvents reported for mosquito larvicidal activity. Its methanol, acetone and benzene extracts reported LC50 values of 515.88, 504.47 and 478.66 ppm against Culex quinquefasciatus respectively [31]; ethanol extract showed activity against Aedes aegypti larvae with LC50 value of 1750 ppm [30], and its hexane and ethyl acetate extracts showed activity against the fourth instar of Anopheles stephensi [32]. Further, Ulva fasciata extracts showed more larvicidal activity when compared with its closely related species, Ulva lactuca, whose acetone extract exhibited LC50 value of 335.30 ppm against Anopheles d'thali [33]; ethanol extract showed activity against Aedes aegypti larvae and LC50 value was 1400 ppm [30], and 0.08, 0.08 and 0.09 µg/mL against Aedes aegypti, Culex quinquefasciatus and Anopheles stephensi [25]; acetone, chloroform, ethanol, methanol and petroleum ether extracts exhibited LC50 values of 5.46, 67.99, 12.82, 27.35, 27.55 mg/mL against the third instar of Culex pipiens [34]; methanol extract reported LC50 values >1000 µg/mL against Aedes aegypti and Aedes albopictus [23]; hexane, chloroform, ethyl acetate, acetone and methanol extracts showed LC50 values of 950.3, 761.6, 588.1, 831.0, 952.0 ppm against Aedes aegypti [27]; acetone, ethanol and petroleum ether extracts exhibited LC50 values of 5.00, 11.70 and 31.69 mg/mL against fourth instar of Culex pipiens [35].

The toxicity of seaweed depends upon the species of seaweeds, the polarity of solvent, and the mosquito species tested. The chemical composition of the seaweed plays an important role in its bioactivity against mosquito larvae. Green seaweeds are prolific producers of metabolites, and their larvicidal

properties might be due to the presence of its effective chemical components like alkaloids, flavonoids, phenolics, saponins, steroids and terpenoids with mosquito larvicidal properties [6,25,29]. Caulerpa species are the most effective green seaweeds due to the presence of terpenoids, and a major secondary metabolite compound caulerpenyne involved chemical defense of genus Caulerpa [14]. Further, alkaloids like caulerpin and caulerpinic acid from Caulerpa racemosa act as insecticidal compounds against the second, third and fourth instar of Culex pipiens and have reported LC50 values of 1.42, 1.81, 1.99 ppm and 3.04, 3.90, 4.89 ppm respectively after 24 hours [36]. These compounds would have been responsible for the larvicidal action in the present study too. On the other hand, genus Ulva contains palmitic and octadecanoic acid, and methyl esters [34]. These chemical compounds are known insecticidal compounds, as they affect the metabolism and morphology of mosquito larvae midgut, especially in Culex quinquefasciatus [37]. Extracts of nonpolar solvents of green seaweeds showed higher insecticidal activity than extracts of polar solvents [38]. However, in the present study, chloroform, a mid-polar solvent exhibited the maximum activity. Bioactive compounds like alkaloids, saponins, and phenolics are extracted by chloroform. Further, chloroform extract of seaweed, Codium edule caused the body of the Aedes aegypti larvae to become longer and dark in colour [39]. The same was observed in the present study also. The susceptibility of mosquito species is too varied [25]. Manilal et al. [40] reported Aedes aegypti larvae were more susceptible when compared to Culex quinquefasciatus on the basis of low LC50 values, and the same was observed in the present study. There was a higher mortality rate for younger larvae compared to older larvae under the same concentration treatment in the present study. Similar observations were reported by Selvin and Lipton [41] wherein the fourth instar larvae were resistant at the concentration that produced 100% mortality in the second instar exposed to green seaweeds.

5. Conclusions

The present study indicated the larvicidal property of the chloroform extract of both the green seaweeds against the vector mosquitoes, which encourages further investigation on its bioactive compounds that might own virtuous larvicidal properties when isolated in pure form may be effective as toxicants against juvenile stages of mosquitoes.

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