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Study of Antioxidant and Larvicidal Properties of Selected Medicinal Plants of Fringe Villages of Manas National Park, Assam, India

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Mosquito-borne diseases are among the major ailments of world affecting billions of people living in economically poor and developing countries. The development of insecticide resistance in mosquito vectors has forced the global community to look into alternative sources of medicines with better efficacy and less side effects. Plants with rich sources of metabolites have been explored extensively for mosquitocidal activity. The present study explored the antioxidant and larvicidal activities of five important plants traditionally used as mosquito repellent by tribal communities of fringe villages of Manas National Park of Assam. Methanolic crude extracts were prepared for all the plants following standard protocols. Phytochemical and antioxidant study was performed following the protocol published in recent publications. Larvicidal bioassay was carried out as per WHO protocol. The study observed considerable phytochemical and antioxidant activity. Phenolics, flavonoid and antioxidant activity, were found to be highest in *Cinnamomum tamala*. The phenolic and flavonoid value ranged from 9.89 to 147.15μgGAE/mg and 4.32 to 28.43μgQE/mg plant extract, respectively. The IC50 for various antioxidant activities ranged from 27.94 to 114.15μg/mL (DPPH), 15.05 to 707.74μg/mL (ABTS) and 40.23 to 338.91μg/mL (TBARS). Similarly, *C. tamala* showed the strongest larvicidal activity with LC50 value of 3.11mg/mL in *Aedes aegypti* larvae. The present study observed that *C. tamala* leaves could be a good source of phytochemicals and antioxidant and larvicidal activity.

Keywords: Antioxidants; Ethnomedicines; Larvicide; Manas National Park; Phytochemicals.

Vector-borne diseases are one of the major diseases that kill nearly one million people every year. Globally, malaria alone results in about 400,000 people death out of about 219 million cases. Dengue is another mosquito-borne disease transmitted by Aedes mosquitoes, affecting nearly four billion people worldwide and causing fatalities of about 40,000 people annually.[1] Though a good number of commercial insecticides are available, the development of drug resistance presents a major challenge to controlling vector-borne diseases.[2] Plants can be an alternative to commercial insecticides with their rich secondary metabolites. Worldwide several plants are being investigated to see the bio-efficacy against many diseases, including larvicidal and mosquitocidal activities.[3] India is among the wealthiest countries with diverse cultural traditions associated with using plants and herbs for human ailments.[4] The use of plants and herbs to cure diseases has been practised in this part of the world since ancient times. Many studies have explored the medicinal

properties of traditionally used plants because of their rich and essential secondary metabolites.[5,6] With rich antioxidant phytocompounds, plants can counteract the toxic and cell-damaging biomolecules, the free radicals.[7-9] In northeast India, people use herbal medicinal formulations to cure common diseases, especially in rural areas. Since ancient times, tribal groups of Assam have been practicing several plant-based preparations to repel insects and mosquitoes from edible items. Based on ethnomedicinal knowledge, the present study investigates the larvicidal activity of five plants commonly used by villages as insect repellents, namely *Azadirachta indica, Brassica rapa, Cinnamomum tamala, Nicotiana tabacum* and *Ocimum sanctum.*

A. indica belonging to the family Meliaceae is an important plant having rich medicinal values. The plant possesses several bioactive properties such as larvicidal, anthelmintic, insecticidal, pesticidal, cancer, hypertension, diabetes, antiviral, anti-inflammatory, anti-dermatic, anti-ulcer, etc.[10-12] *B. rapa* is a herbaceous plant belonging to the family Brassicaceae. Traditionally, the plant parts are used for numerous ailments such as rheumatism, neuralgia, alopecia, snakebite, toothache, carcinoma, throat tumours, bronchitis, cardiovascular disease, and diabetes.[13,14] *C. tamala* (Family Lauraceae), also known as Tejpatt in Assam, is an evergreen, aromatic tree distributed widely in north-eastern Himalayas in Assam, Mizoram, and Meghalaya. Traditionally, different parts of *C. tamala* are used to treat cough, asthma, wound healing, food preservatives, and spices.[15,16] Similarly, *N. tabacum* is a herbaceous plant (Family Solanaceae) known to enhance digestive system, urinary tract disorders, cough, itching, helminth-related problems, toothache, pain in the eye, scorpion bite, relaxant, and antispasmodic.[17] *O. sanctum* (Family Lamiaceae) is a herbaceous plant used to treat snake bites, scorpion stings, skin infections, cough, respiratory disorders, malarial, wound healing, and ulcers.[18,19] Because of its medicinal importance, the present study investigated the antioxidant and larvicidal properties of five traditionally used medicinal plants, *Azadirachta indica, Brassica rapa, Ocimum sanctum*, *Cinnamomum tamala,* and *Nicotiana tabacum*.

METHOD AND MATERIALS

Collection and identification of plant materials

Five medicinal plants, namely *Azadirachta indica* A. Juss. (identification no. BUBH2118051), *Brassica rapa* L. (BUBH0000849), *Ocimum sanctum* L. (BUBH28045), *Cinnamomum tamala* (Buch-Ham) T.Nees & C.H. Ebern (BUBH0000860), and *Nicotiana tabacum* L. (BUBH0000861) were collected from fringe villages of Manas National Park (N-26°45′17.3′′ E-091°13′57.3′′), Assam, India. The scientific identification of plants was carried out in the Department of Botany, Bodoland University, Kokrajhar.

Preparation of alcoholic crude extract

Sample plants were collected from the fringe villages of Manas National Park, Assam. The leaves were processed for alcoholic crude extract preparation following the process described in earlier publications.[20,21] Dry or semi-solid extracts of plants were collected and kept in a vial at -20ºC for further experimental uses.

Phytochemical Study

Qualitative study

The qualitative phytochemical of methanolic plant extract was determined by the following latest publications.[22,23] The various qualitative tests performed were as follows: Tannins (Braymer's test), Saponins (Frothing test), Terpenoids (Salkowski test), Reducing Sugar (Fehling's test), Coumarins (NaOH test), Quinones (Sulphuric acid test), Alkaloids (Dragendorff test), Phlobatannins (HCl test), Anthraquinones (Borntrager' test), Anthocyanin (HCl test), and Cardiac Glycosides (Keller-Kiliani test).

Quantitative study

Carbohydrates

Anthrone method was used for the estimation of crude carbohydrate content of crude plant extracts.[24] The value (µg/mg plant extract) was correlated with the standard curve prepared using glucose ($R^2 = 0.9741$).

Protein

Lowry method was used for the estimation of protein using Folin-Ciocalteu reagent.[25] Values were expressed as µg protein/mg plant extract using the calibration curve of BSA ($R^2 = 0.999$).

Total Phenolic Content (TPC)

The presence of total phenolic contained

was evaluated by Folin-Ciocalteu reagent.[26] TPC value was compared as gallic acid equivalent (R^2 = 0.995).

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was estimated following the method of Ordonez et al.[27] Quercetin was used as a reference chemical for standard curve preparation (R^2 = 0.9879).

Antioxidant Activities

Total antioxidant activity (TAA) assay

TAA of plant extract was estimated following phosphomolybdate method.[28] TAA value was compared with ascorbic acid as the standard chemical.

Ferric-reducing antioxidant power (FRAP) assay

The procedure of Iloki-Assanga et al.[29] was followed to estimate FRAP activity of plant extracts. FRAP activity of the plant extract was compared with the ascorbic acid (as standard).

1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) assay

The antioxidant activity of plant extracts was estimated by the scavenging activity of DPPH free radicals following Mamta et al.[30] The scavenging activity was measured as percentage inhibition using the following formula:

$$\text{Percentage inhibition} = \left[\frac{Abs\ control - Abs\ sample}{Abs\ control}\right] \times 100 \quad ....(1)$$

Abs control = absorbance of DPPH and methanol. Abs sample = absorbance of DPPH and plant extract or ascorbic acid.

2-2'-Azinobis- (3-ethylbenzothiazoline-6-sulfonate) (ABTS) assay

The free radical scavenging activity of plant extracts was also estimated using ABTS as free radicals.[31] The ABTS radical scavenging activity of plant extract was compared with gallic acid as a standard antioxidant molecule. The free radical scavenging potentials of the plant extracts were estimated following the formula of DPPH assay.

Lipid peroxidation inhibition or Thiobarbituric acid reactive species (TBARS) Assay

Lipid peroxidation inhibition property of plant extracts was studied in egg yolk as a lipid-rich medium following the modified protocol of Thiobarbituric acid reactive species.[32] Ascorbic acid was used as a reference chemical. The formula for the calculation of TBARS was similar to DPPH assay.

The larvicidal bioassay

Larval bioassay was carried out as per WHO protocol. Larvae were exposed to different test doses of plants as per the efficacy of the plants. The overall test doses ranged from 3.0 – 12 mg/mL for different plant extracts. A total of 20 - 25 numbers of 3rd or 4th instar *A. aegypti* larvae were exposed to different plant extract concentrations for 24h.[33] The experiment was replicated three times.

Statistical analysis

Statistical calculations were performed in Mis. excel and OriginPro-8.5. Correlation study was carried out in IBM-SPSS-Ver.21. All

Table 1. Qualitative analysis of phytocompounds from five medicinal plants

Phytochemicals Test performed	Name of the plants *O. sanctum*	*C. tamala*	*N. tabacum*	*B. rapa*	*A. indica*
Tannins	+	+	-	-	+
Saponins	+	+	-	-	+
Coumarins	+	-	+	+	+
Quinine	+	+	+	+	-
Alkaloids	-	+	+	+	-
Terpenoid	+	+	+	+	+
Phlabotannins	-	+	+	+	-
Reducing sugar	-	-	-	+	-
Anthracyanine	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Glycosides	+	-	-	+	+

"+" indicate present, "-" indicate absent

experiments were carried out in triplicate (n=3), and values were expressed as mean ± standard deviation (SD).

RESULTS

Plants have been used as emerging sources of medicines in the modern-day scenario as they are rich in phytochemicals and secondary metabolites. Table 1 shows the qualitative analysis of phytocompounds. Fig. 1 shows the protein, carbohydrate and phytochemical contents of all five plants. The study observed a considerable quantity of protein content in the methanolic extracts of plants, while the carbohydrate content was much higher (almost five times) than protein. The average protein and carbohydrate content were found to be 78.68±8.26µg/mg and 355.80±115.57µg/mg plant extract, respectively. Highest protein content was revealed in *O. sanctum* (86.67±4.64 µg/mg extract) and lowest in *A. indica* (67.07±6.01µg/mg extract). On the contrary, *A. indicum* and *O. sanctum* showed highest (564.52±8.76µg/mg extract) and lowest (222.04±3.98µg/mg extract) carbohydrate content, respectively (Fig. 1a). The crude extracts of plants showed significant variations in phenolic and flavonoid contents among the five plants. Highest TPC and TFC was found in *C. tamala*. Lowest TPC and TFC were found in *B. rapa* and *A. indicum*, respectively. The TPC ranged from 9.89 (*B. rapa*) to 147.15µgGAE/mg extract (*C. tamala*). Overall, the phenolic content was significantly higher (average, 55.25µgGAE/mg extract) compared to flavonoid content (average, 16.78 µgQE/mg extract). TFC value ranged from 4.32 to 28.43µgQE/mg extract (Fig. 1b). Similarly, *C. tamala* showed strongest total antioxidant and ferric-reducing properties among the five plants, while *B. rapa* and *N. tabacum* showed weakest total antioxidant and FRAP activity, respectively. Among the five plants, the total antioxidant activity ranged from 210.47±2.06 to 397.61±4.23µgAAE/mg extract, respectively. Similarly, *C. tamala* showed highest FRAP activity, while *N. tabacum* showed the lowest ferric-reducing activity (Fig. 1c).

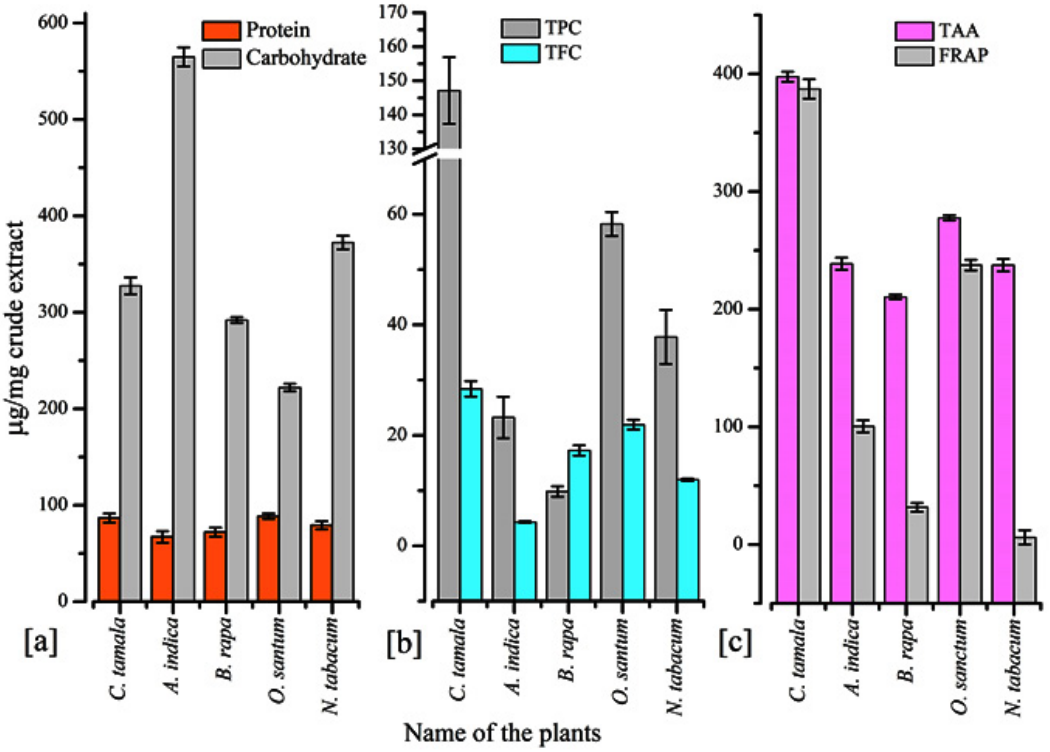

Fig. 1. Phytochemical contents of plants. (a) total protein and crude carbohydrate, (b) phenolic and flavonoid content and (c) total antioxidant and ferric-reducing activity of plants

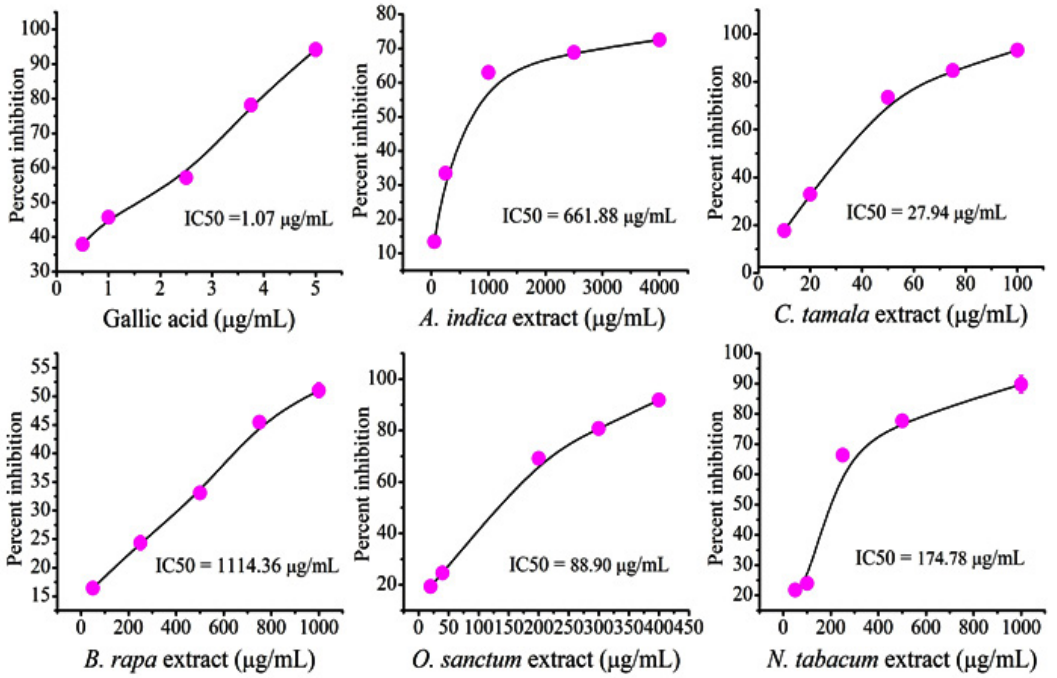

Fig. 2. DPPH free radical scavenging activity of plants

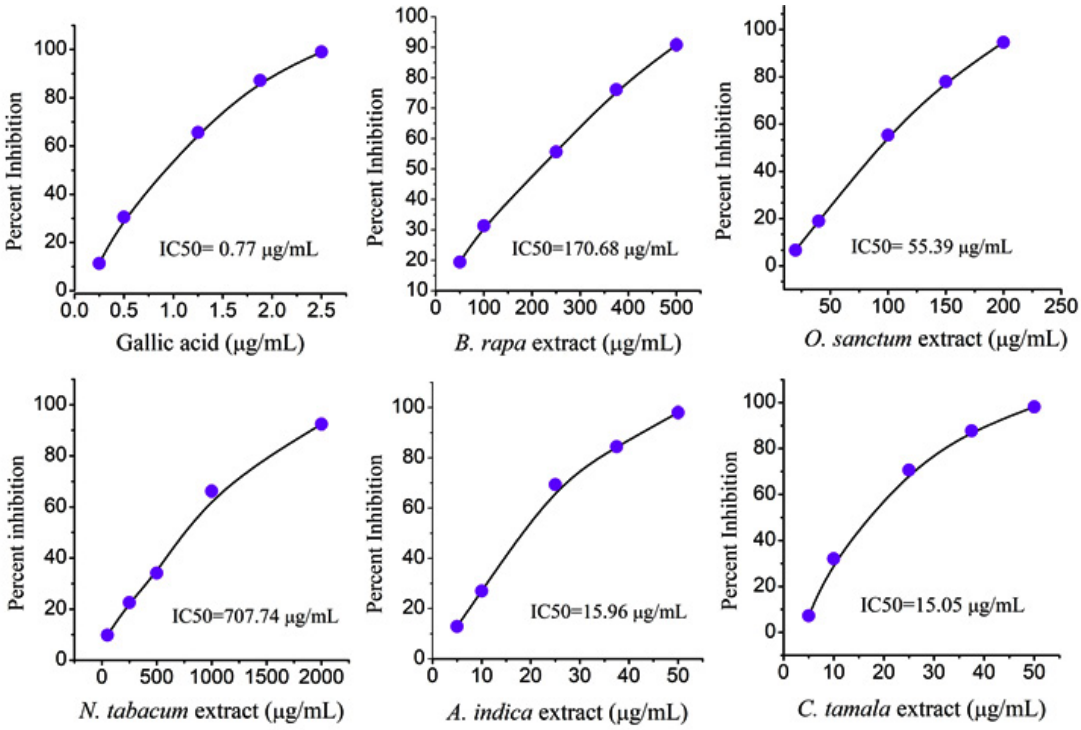

Fig. 3. ABTS free radical scavenging activity of plants

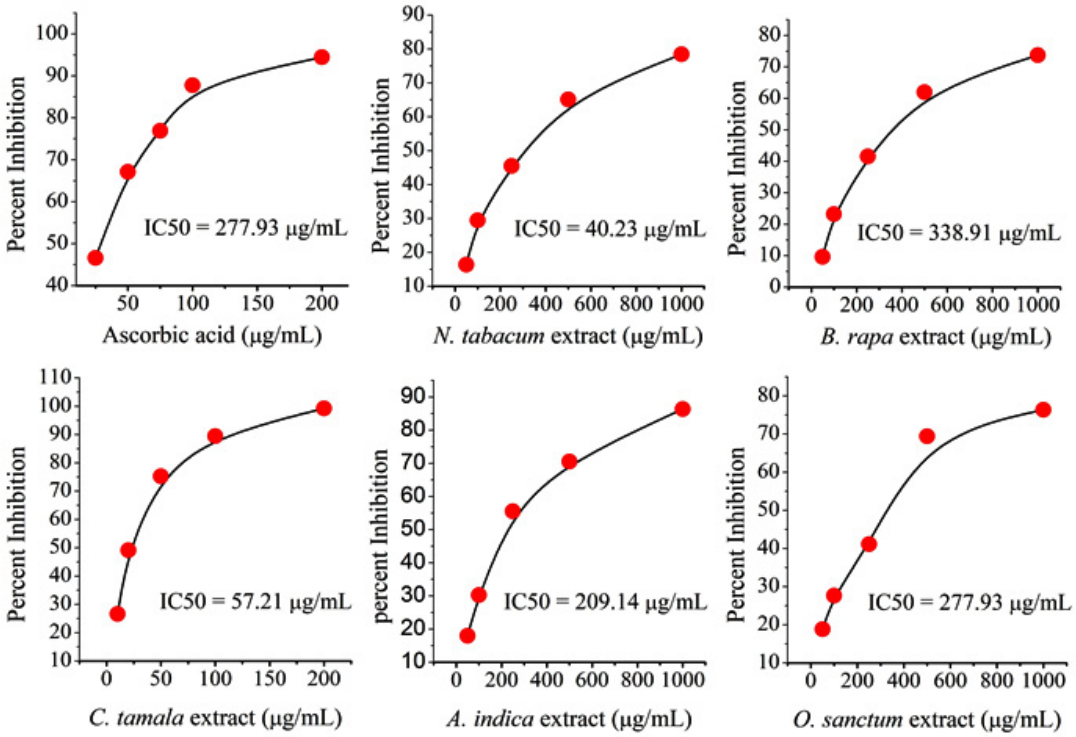

Fig. 4. Lipid peroxidation inhibition activity of medicinal plants

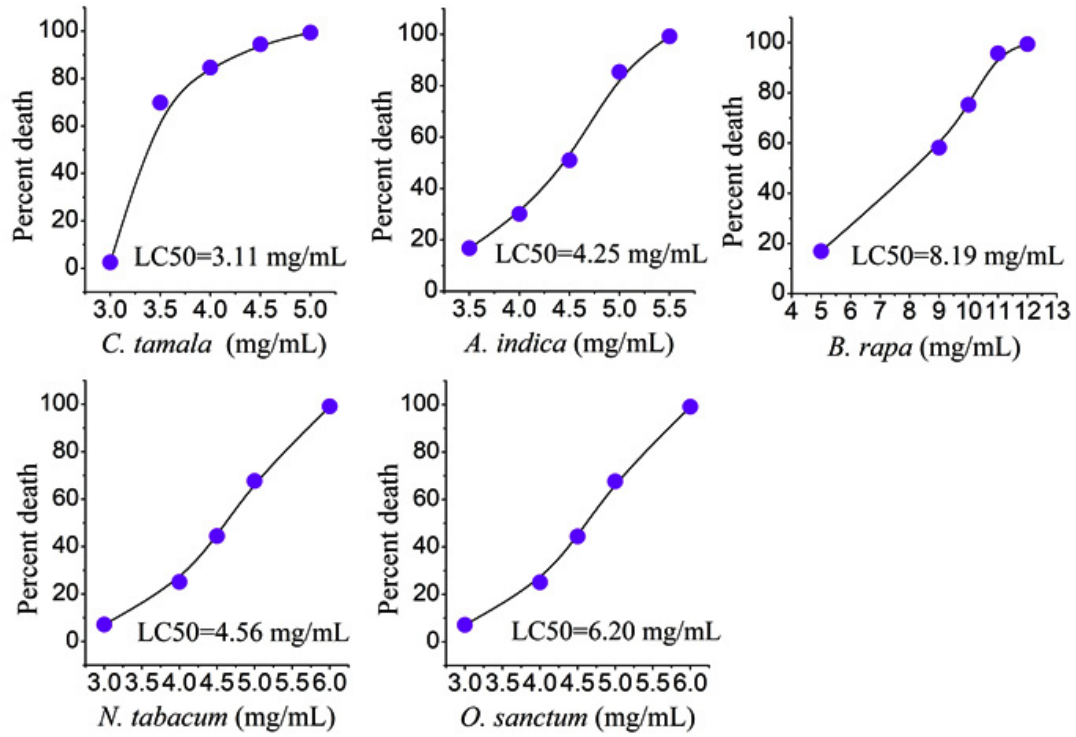

Fig. 5. Larvicidal activity of medicinal plants against *Aedes aegypti* mosquito

The present study explored the antioxidant properties of plant extracts. All the plants showed concentration-dependent antioxidant activity. Fig. 2 shows the DPPH free radical scavenging activity of all the plants. Overall, *C. tamala* showed the most active scavenging activity of DPPH free radicals. Increased concentration of plant extracts showed increased DPPH scavenging activity. Highest DPPH radical scavenging activity was found in *C. tamala* (IC_{50}, 27.94µg/mL), followed by *O. sanctum* (IC_{50}, 88.90µg/mL) and *N. tabacum* (IC_{50}, 174.78µg/mL). About 94% inhibition was observed in 100µg/mL of *C. tamala* crude extract. *B. rapa* (IC_{50}, 1114.36µg/mL) showed the lowest DPPH free radical scavenging activity, while the reference chemical, ascorbic acid, showed strongest antioxidant activity with an IC_{50} value of 1.07 µg/mL. The average IC_{50} value for all five plants was found to be 413.57µg/mL.

For ABTS assay, both *C. tamala* and *A. indica* showed the strongest ABTS free radical scavenging activity with almost similar IC_{50} values of 15.05 and 15.96µg/mL, respectively (Fig. 3). Unlike DPPH assay, *N. tabacum* showed the weakest ABTS radical scavenging property (IC_{50}, 707.74µg/mL). All the tested plants showed much stronger ABTS radical scavenging activity than DPPH. The mean IC_{50} value for ABTS assay was 192.96µg/mL, almost two times smaller than the DPPH IC_{50} value. The reference chemical, ascorbic acid, showed the most potent ABTS scavenging activity with an IC_{50} value of 0.77µg/mL.

Plants are also known to possess chemicals that exhibit inhibition properties of lipid peroxidation inhibition. The present study also observed dose-dependent lipid peroxidation inhibition properties in all the plants. *N. tabacum* showed the most potent peroxidation inhibition property among the five plants with IC_{50} value of 40.23µg/mL, followed by *C. tamala* and *A. indica*. Alternatively, *B. rapa* showed the weakest inhibition property of lipid peroxidation (IC_{50}, 338.91µg/mL). The average IC_{50} value for lipid peroxidation inhibition of plant extracts was found to be 184.68µg/mL (Fig. 4).

Fig. 5 shows the larvicidal activity of all five plants. Like antioxidant activity, the study observed dose-dependent mortality of the larvae after 24 h treatment. Of five plants, *C. tamala* showed the most potent larvicidal activity. *A. indica* and *N. tabacum* also showed almost similar larvicidal activity. The LC_{50} value ranged from 3.11 mg/mL to 8.19 mg/mL, with a mean value of 5.26 mg/mL.

DISCUSSION

Plants have been the source of phytocompounds and secondary metabolites having rich medicinal values and are investigated for several medicinal values. They are preferably the second most chosen treatment system against synthetic drugs and have attracted scientific attention since the 19th century. Based on ethnomedicinal values, the present study investigated the phytochemical, antioxidant, and larvicidal properties of five ethnomedicines, namely - *C. tamala*, *A. indica*, *N. tabacum, B. rapa* and *O. sanctum*. The present study observed that the plant extracts contain substantial phytochemical content and antioxidant properties. Many studies have reported similar results having rich phenolic and flavonoid content in several plants.[34,35] Phenolics are widely distributed secondary metabolites in plants with tremendous medicinal values and health benefits. The present study observed considerable quantities of phenolics and flavonoids in all the plants. Highest quantity was reported in *C. tamala* (147.15 µgGAE/mg extract). In the same way, Bernard et al.[36] showed similar results in *Cinnamomum zeylanicum* and *C. osmophloeum* leaf extracts. On the contrary, Rahman et al.[37] showed a significant difference in phenolic content (2.76mgGAE/g) compared to our result. Silva et al.[38] also revealed a considerably lower quantity of phenolic content (8.08±1.83mgGAE/g) in the ethanolic extract of *C. triplinerve* leaves.[38] Presence of phenolics and flavonoid contents is linked to the antioxidant property of plants. In the present study, we observed a significant relationship between phenolics and the antioxidant property of plants. Our study showed highest antioxidant activity in *C. tamala* among the five plants. Following our study, Prasad et al.[39] also reported a very relevant result in *C. zeylanicum*. Abeysekera et al.[40] also showed the leaf and barks of *C. zeylanium* to possess strong antioxidant activity comparable to our result. The IC_{50} values of antioxidant study ranged from 27.94 µg/mL to 1114.36 µg/mL for DPPH study averaging 413.57 µg/mL for all five plants. In a

similar study, several researchers have revealed almost similar antioxidant properties of *C. tamala* and *C. verum,* as reported in the present study.[41,42]

Like the antioxidant study, our study also revealed potential larvicidal properties of all five plants. In the same way, Das et al.[43] also showed that the plant roots of *Andrena saccata* and leaves of *A. squamosa* had the most larvicidal activity against *Culex quinquefasciatus* and *Ae. aegypti* larvae with methanol and ethanol extract. A very similar kind of study and findings were seen in Iqbal et al.[44] with *C. tamala* and *O. basilicum* leaves, where LC_{50} value of *C. tamala* was 1.48mg/mL, and *O. basilicum* was found to be 5.32mg/mL on *Cu. quinquefasciatus*.[44] Ullah et al.[45] also showed the larvicidal efficacy of *Nicotiana tabacum* with LC_{50} value of 17.77ppm in *Cu. quinquefasciatus* of 3rd instar larvae were observed in their study. Similarly, the aqueous extract of *A. indica* seed was investigated, and it found that doses up to 1.0 – 5.0mg/mL have strong larvicidal activity with almost 77 - 99% mortality of *Cu. quinquefasciatus*.[46] Komolamisra et al.[47] tested 84 ethnomedicinal plants of Thiland, where *C. rhyncophyllum* showed considerably better larvicidal activity with an LC_{50} value of 188.64 mg/mL. The presence of high phenolic content and strong antioxidant molecules might have improved the larvicidal property of *Cinnamomum tamala* on *Aedes aegypti* larvae showing the most effective larvicidal activity compared to other plants. However, further study needs to be carried out to explore the bioactive phytocompounds responsible for larvicidal activity and the molecular mode of action.

CONCLUSION

Traditional uses and ethnomedicinal values of the fringe villages of Manas National Park of Assam, India, were the primary basis of the present study. The study observed that traditional ethnomedicinal practices have a scientific background and merit. The present study approves the traditional faiths behind the insecticidal properties of *A. indica, B. rapa*, *O. sanctum*, *C. tamala,* and *N. tabacum*. With its potent larvicidal activity supported by the presence of rich phenolics, flavonoids, and antioxidant properties, the present study opined that the leaves of *C. tamala* could be a potential source of insecticidal agents, and further studies may be carried out to explore the molecular mode of action.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest among the authors.

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