Mapana Journal of Sciences
2022, Vol. 21, No. 4, <pages>
ISSN 0975-3303 |https://doi.org/10.12723/mjs.63.article#

Studies on Phytochemical Analysis, Antioxidant, Antibacterial and Larvicidal Properties of the *Acacia nilotica* Fruit Extracts

Niloy Das , Sonali Saha , Utpal Singha Roy*

Abstract

In the present study fruit of *Acacia nilotica* was chosen to evaluate its antioxidant, larvicidal and antibacterial properties. Phytochemical screening of aqueous and alcohol extracts of the plant fruits affirmed the presence of carbohydrates, reducing sugar, phenol, flavonoid, terpenoid, saponin and steroid. The antioxidant properties of the aqueous and alcohol fruit extracts were noted as 10.11±0.035 and 9.75±0.023 mM of $FeSO_4$ for Ferric Reducing Antioxidant Power (FRAP) assay respectively. The potency of antimicrobial activity of the aqueous and alcohol extract of *A. nilotica* fruit were tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillussubtilis*, and *Escherichiacoli*. The alcohol extract against *E. coli*, exhibited the highest antimicrobial activity. Aqueous extracts of *A. nilotica* fruit showed potential toxicity against *Aedes albopictus* larvae with LC_{50} value of 142.074 mg L^{-1}. Present findings clearly indicated that *A. nilotica* fruit extracts could be most effectively used as a natural antioxidant, antibacterial and larvicidal agent.

Department of Chemistry, P. R. Thakur Govt. College, Thakurnagar, West Bengal, India; niloy.prof@gmail.com
Department of Microbiology, P. R. Thakur Govt. College Thakurnagar, West Bengal, India; sahasonalee12@gmail.com
* Department of Zoology, P. R. Thakur Govt. College, Thakurnagar, West Bengal, India; srutpal@gmail.com (* Corresponding Author)

Keywords: *Acacia nilotica*; *Aedes albopictus*; Aluminum chloride assay; Antimicrobial activity; Antioxidantassay; Mosquito larvicide bioassay

1. Introduction

Phytochemicals are secondary metabolites that perform different roles including antioxidant, larvicidal and antimicrobial agents, and are in use for the betterment of mankind. Medicinal plants are the richest bioresource of phytochemicals which contain a complex mixture of alkaloids, glycosides, terpenoids, flavonoids, and steroids. Various general techniques such as percolation, digestion, decoction, hot continuous extraction, and maceration are used along with solvents of different polarity to extract these medicinal active components from different parts of plants. The quantification and composition of phytochemicals depend on extraction technique, estimation time, temperature, nature of the solvent, and solvent concentration [1].These secondary metabolites show many pharmaceutical activities for example terpenoids exhibit anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities [2], and alkaloids are used as anaesthetic agents[3]. Since the plant diversity of India is very rich there is enormous scope for screening and characterization of the phytochemicals extracted from various plant species for a wide range of use to improve human health.

Mention may be made that in the last few decades, environmental pollution has been recognized as a big concern for human health. Environmental pollution along with ionizing radiation and other physiological causes like metabolism generates Reactive Oxygen Species (ROS) in living organisms(ROS are singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite). Excess of ROS leads to oxidative stress such as the deactivation of many enzymes and cell damage by irreversible oxidation of DNA, proteins, and lipids[4, 5]. Antioxidants play an important role in this regard to protect cells from such reactive oxygen species by scavenging them from the body. The mechanism of action of antioxidants is very simple. They actually act as a reducing agent which blocks the chain reaction of free radicals by supplying the hydrogen ion. Synthetic antioxidants are hazardous to human health. So, the search for natural antioxidants is very necessary, and

medicinal plants are good reservoirs of natural antioxidants. Various secondary metabolites for e.g., phenol, flavonoids, and amino acids, are the main compounds that act as natural antioxidants[6]. *Acacianilotica* has been previously reported as a potential source of antioxidant polyphenols like tannin, gallic acid, ellagic acid, and catechin [7].

Apart from the reactive oxygen species (ROS), various pathogens also have deleterious effects on human health. Since the discovery of Penicillin, antibiotics have been used to treat infectious diseases. Considering the fact that a huge number of antibiotics have already been discovered, abuse (as a growth factor in animal farms, prophylactic and self-medication by nonmedical individuals) and overuse of those antibiotics has become one of the prime reasons for the rise and spreading of resistant strains commonly, multi-drug resistant strains (MDR) or extensively drug-resistant (XDR) of numerous groups of microorganisms [8]. The other considerable reasons are excessive use of immunosuppressive drugs, organ transplantation, adulterations in chemotherapeutic agents, and improper use of medical equipment, etc. The previous findings of the extensive occurrence of antibiotic resistance to freshly presented antibiotics indicate that even new generations of antibiotics will have a petite life prospect [9]. Thus, antimicrobial resistance is now considered a serious global problem, increasing mortality and morbidity. Recent studies have shown that the clinical isolates of various common human pathogens such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeruginosa* swiftly gain antibiotic resistance and rapidly spread in the environment [10]. Therefore, now the world needs alternative and preferably natural compounds or even technics having antimicrobial activity. Since ancient times, several plant products have been used as medicine. In some previous studies, it was found that various medicinal plant extracts have potential antimicrobial activity against harmful microorganisms. Aqueous extracts of *Acacia nilotica*, *Justicia zeylanica*, *Lantana camera*, and *Saracaasoca* have been reported to possess antimicrobial activity against some selective bacteria [11].

World Health Organization, in the year 2020, had published a list of the thirteen most urgent human health challenges that needed to be addressed over the next ten years [12]. Along with antibiotic

resistance due to the overuse of antibiotics, the list included the spread of mosquito-borne infectious diseases like malaria and dengue [12]. Considering human health, mosquitoes are still considered the deadliest enemy. Mosquitos are tiny assassins of nature that kill more than a million victims around the world in a single year [13]. Their annoying presence is felt in the diseases they manifest, including malaria, filariasis, dengue, yellow fever, and encephalitis [14]. Hazardous chemical pesticides not only cause human immune dysfunction, various forms of cancers, and birth defects but also potentially perturb various components of the ecosystem [15, 16]. Actually, these chemical pesticides have enough potential to destroy major habitat types around the globe along with the adverse effects on agriculture [17]. The need of the hour is thus to find suitable eco-friendly alternate sources of larvicides that can actively control mosquito menace and be gentle to nature at the same time [18]. Medicinal Plant extracts, which are natural products, are considered excellent in this regard [19].

One such medicinally important plant is *Acacia nilotica* which belongs to Fabaceae family of genus *Acacia and* is commonly known as 'babla'. The plant is native to the Middle East and the Indian subcontinent. Different parts of *Acacia nilotica* have been used traditionally to treat wounds, pharyngitis, bronchitis, diabetes, wound ulcers, skin disease, tuberculosis, and smallpox[20]. Literature also suggests that *Acacia nilotica* has health-giving power against different microbial diseases, hypertension, and haemorrhoid [20]. Considering the previous facts obtained from various kinds of literature, in the present study, the medicinal plant *Acacia nilotica* was chosen to assess its antioxidant, antimicrobial, and larvicidal effects.

2. Materials and Methods

2.1 Plant Material

The fruit of *Acacia nilotica* was collected from the campus of Durgapur Govt. College. This plant was identified botanically in the Department of Botany, Durgapur Government College, Durgapur-14, Paschim Bardhaman, West Bengal. The fresh fruit was thoroughly rinsed in water and shade dried at room temperature. The dried fruits were finely ground in a blender and stored in an air

tight container box at room temperature. All the analysis were done within six months of collection of plant samples.

2.2 Reagents

Ethanol, methanol, distilled water, aqueous HCl, chloroform, concentrated sulphuric acid, Ammonia solution, picric acid, bismuth nitrate, potassium iodide, ferric chloride, α-napthyl amine, sodium hydroxide, aluminium chloride, potassium acetate, Folin-Ciocalteu reagent, gallic acid, qercetin, TPTZ, potassium ferrocyanide, ferrous sulphate, agar, and other chemicals were of the highest purity available. All these chemicals were procured from different reputed companies like Sigma-Aldrich, Merck India, Himedia, and SRL.

2.3 Preparation of Aqueous and Alcohol Extracts

The aqueous extract of *Acacia nilotica* fruits was prepared by following the maceration process [21]. 5 gm of finely ground plant powder was taken in a 100 ml beaker, and 50 ml sterile distilled water (1:10 w/v) was added to it. Then it was stirred for 5 hrs on a magnetic stirrer and soaked for 24 hrs at room temperature. After that, the water extracts of different plants were filtered through a cotton filter, and the filtrates were collected in sample vials. After that, it was centrifuged at 1500 rpm for 10 minutes and the supernatant was collected. Again, these were re-filtered by using Whatman Filter No. 1 to obtain a clear solution. This formed the stock solutions which were stored in the refrigerator at 4^0C till further use.

The alcohol (ethanol) extract of plant powder was also prepared by the above-said method.

2.4 Qualitative Analysis of Phytochemicals

The aqueous and ethanolic solvent extracts of *Acacia nilotica* plant were subjected to different chemical tests to screen the phytochemicals. The tests have been described in the following section.

2.4.1 Test for phenol

$FeCl_3$ test: 2-3 drops of 10% aqueous $FeCl_3$ (w/v) solution were added to the plant extract. This test was done in triplicate. The emergence of blue-green colour confirmed the presence of phenol [21].

2.4.2Test for tannins

KOH test: To the 1ml plant extract 1 ml of freshly prepared 10% KOH was added. This test was done in triplicate. Dirty white precipitates indicated the presence of tannins [22, 23].

2.4.3Test for carbohydrates

Molisch test: The plant extract was treated with 2-3 drops of Molisch's reagent (10% of 1-napthol in ethanol). After this, a small amount of conc. H_2SO_4 was added carefully along the sides of the test tube. This assay was done in triplicate. The presence of carbohydrates was confirmed by observing the formation of a reddish-violet ring at the junction.

2.4.4Test for reducing sugars

4 ml of Fehling solution was prepared by mixing Fehling A and B in equal amounts. To this solution, 1ml of plant extract was added and boiled for 5-10 minutes. This assay was done in triplicate. Brownish red precipitates indicated the presence of reducing sugars.

2.4.5Test for flavonoids

NaOH test: 3ml plant extract was treated with 1ml of 10% aqueous NaOH solution. This test was done in triplicate. The appearance of intense yellow colour, which turned colourless on the addition of dilute HCl indicated the presence of flavonoids.

2.4.6 Test for saponins

Froth test: 2ml of plant extract was taken in a test tube and it was shaken vigorously and observed for froth formation [24]. This assay was done in triplicate.

2.4.7Test for steroids

H_2SO_4 test: To the 1ml of plant extract, a few drops of conc. H_2SO_4wereadded from the side walls of the test tube. This test was done in triplicate. The appearance of red colour indicated the presence of steroids [24].

2.4.8 Test for terpenoids

Salkowski test: The plant extract was mixed with twice the volume of chloroform and then 3ml of conc. H_2SO_4 was added carefully from the sides of the test tube. This assay was done in triplicate. Reddish brown colouration at the interface indicated the presence of terpenoids [22].

2.5 Quantitative Estimation of Phenol and Flavonoid

2.5.1 Estimation of Phenol Content

The quantitative estimation of simple phenol was done by the Folin-Ciocalteu assay method [25]. 1 ml of aqueous and alcoholic extracts of *A. nilotica* were mixed separately with 9 ml of distilled water and 1 ml of Folin-Ciocalteu phenol reagent in a volumetric flask (25 ml). After 5 minutes, 10 ml of 7% Sodium carbonate (Na_2CO_3) solution was treated to the mixture and incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Equiptronics visible scanning single beam spectrophotometer (EQ-822A). Solutions of gallic acid were used to prepare a standard curve. This measurement was done in triplicate. The phenol content was expressed as mg of GAE/ml of the extract [26].

2.5.2 Estimation of Flavonoid content

The aluminum chloride method was used for the determination of the total flavonoid content.1 ml of aqueous and alcoholic extracts of *A. nilotica* was mixed separately with 4 ml of distilled water and incubated for 5 minutes with 0.30 ml of 5% sodium nitrite in a different 10 ml volumetric flask. After that mixtures were incubated with 0.3 ml of 10% aluminum chloride for another 5 minutes. Finally, 2 ml of 1M Sodium hydroxide was added and diluted to 10 ml with distilled water. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an Equiptronics

visible scanning single beam spectrophotometer (EQ-822A). Quercetin solution was used to prepare the standard curve. This measurement was done in triplicate. The flavonoid content was expressed as mg of QE/ml of the extract[27].

2.6 Tests for Antioxidant Property

2.6.1 Reducing Power Method

Here, the antioxidant power was measured in terms of reducing the ability of the sample. Suitable method was followed, as described earlier[28]. 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml $K_3Fe(CN)_6$ (1% w/v) were added to 0.5 ml of *A. Nilotica* plant extract dissolved in 0.5 ml distilled water. The resulting mixture was incubated at 50^0C for 20 min, followed by the addition of 2.5 ml of Trichloro acetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5ml), mixed with 2.5 ml distilled water and 0.5 ml 0.1%(w/v)$FeCl_3$. The solutions of ascorbic acid were used for the preparation of the standard curve. The absorbance was then measured at 700 nm against a blank sample. This antioxidant assay was done in triplicate.

2.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

This antioxidant assay was conducted following the standard method [29]. Fresh FRAP solution was prepared by mixing 25 ml acetate buffer, 2.5 ml 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ), and 2.5 ml $FeCl_3.6H_2O$ solution. 2.85 ml of freshly prepared FRAP reagent was mixed with 150 µl of aqueous and alcoholic extracts of *A. Nilotica* separately and incubated at 37^0C for 30 min in the dark. The absorbance was recorded at 593 nm using an Equiptronics visible scanning single beam spectrophotometer (EQ-822A). This antioxidant assay was done in triplicate.The standard curve was prepared for $FeSO_4$ (0.2 mM to 1 mM) solution to express the antioxidant power of plant extract in mM Fe(II)/g dry powder.

2.7 Antimicrobial Activity

The antimicrobial activity of *Acacia nilotica* was studied with aqueous and alcohol extracts on four bacterial strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*.

2.7.1 Collection and Maintenance of Bacterial Cultures

Bacterial isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* were collected from the stock cultures of Microbiology Laboratory, Microbiology Department, Vijaygarh Jyotish Ray College, (8, 2, Jadavpur Central Rd, Bijoygarh, Jadavpur, Kolkata, West Bengal 700032). Nutrient Agar slants of those bacterial cultures were maintained by periodic sub-culturing technics and stored at 4°C.

2.7.2 Inoculum Preparation and Standardization

Mueller Hinton Agar (MHA) used for direct sensitivity testing was prepared following the protocol given by the manufacturer: 38 g medium was dissolved in 1L of distilled water and autoclaved at 121 °C for 15 minutes. By following aseptic conditions in laminar airflow, the medium was poured into 90 mm sterile agar plates and left to solidify. Those plates were incubated overnight at 37 °C, and as no growth occurred after, the plates were considered sterile and thus used for the next steps.

Each bacterial broth culture was prepared and standardized. First of all, the Mueller-Hinton Broth (MHB) in distilled water was prepared and autoclaved. Four/five well-isolated colonies with the same morphological characteristics of each bacterium were peaked up by sterile toothpick from fresh nutrient agar plates of bacterial culture and aseptically transferred into test tubes containing 5 mL of nutrient broth and incubated for about 6 hours. When the turbidity of each bacterial culture reached 0.08 O.D. at 600nm, within 15 minutes, it was used.

(The turbidity of the inoculum tube was adjusted to the already prepared turbidity equivalent of 0.5 McFarland standard, which is assumed to contain a bacterial concentration of 1×10^8 CFU/mL.)

2.7.3 Agar Diffusion Assay

The standardized cultures of each bacteria sample were evenly spread over separately on the surface of Mueller Hinton Agar plates by surface spreading using a sterile glass spreader. The sensitivity testing of the plant extracts was done using the agar well diffusion method whereby, wells of 9 mm diameter and 5 mm depth were made on the solid agar using a sterile metal borer. About 50 µl of the

alcohol extract, of the concentration of 1 g/ml, was dispensed into respective wells, and 20 µg/ml Tetracycline and Streptomycin were used as a positive control since they have a broad spectrum. Sterile 1% K_2HPO_4 (in distilled water) was used as a negative control. After the addition of plant extracts, antibiotics, and 1% K_2HPO_4 each plate was allowed to stand at 4 °C for 10 minutes to permit pre-diffusion of the extract to the MHA medium. Tests were repeated in duplicates to obtain better results. The test plates were kept at 37 °C and were incubated for 24 hours. Twenty-four (24) hours later, the zones of inhibition were measured using a ruler and a pair of dividers, and results were reported in millimetres (mm). The well diameter (9 mm) was deducted from each data of zone of inhibition. This assay was done in triplicate. The mean zone of inhibition and their SD value was calculated for each plant extract and standard antibiotic(Table 2).

2.8.1 Larvae Collection and Maintenance

Aedesalbopictus larvae were collected from adjacent areas of P. R. Thakur Government College (P. R. Thakur Sarani, Thakurnagar, North 24 Parganas, West Bengal 743287). After careful transport to the laboratory, they were reared in dechlorinated tap water and fed with fish feed.

2.8.2 Larvicidal Studies

Mosquito larvicidal bioassays were performed following the standard protocol, "guidelines for laboratory and field testing of mosquito larvicides" suggested by the World Health Organization[30] with slight modifications. Aqueous plant extracts were prepared as per the method already described in section 2.3. Stock solution for the larvicidal study was taken as 10000 mg L^{-1} of *Acacia nilotica* fruit (w/v). Dechlorinated tap water was used to serially dilute the stock solution to get 12 different concentrations of test samples. These were 10000 mg L^{-1}, 5000 mg L^{-1}, 2500 mg L^{-1}, 1000 mg L^{-1}, 500 mg L^{-1}, 300 mg L^{-1}, 200 mg L^{-1}, 100 mg L^{-1}, 50 mg L^{-1}, 25 mg L^{-1}, 10 mg L^{-1} and 5 mg L^{-1}. Dechlorinated tap water was used as a control. 25 larvae of late 3rd and early 4th instars of *Aedesalbopictus* were carefully transferred to beakers of 250 ml capacity containing the test samples of 100 ml. Three such replicates were made for having a better idea of larvicidal bioassays. Beakers were kept at

room temperature (35±2 ºC) and after 24 hours mortality of mosquito larvae was noted. A larva was considered dead if it settled at the bottom of the beaker and didn't make any movement when touched by a needle. The data obtained for larval mortality from the three replicates were averaged and subjected to probit analysis for calculating LC_{50}.

3. Results and Discussion

3.1 Qualitative Analysis of Phytochemicals

The qualitative analyses were performed using aqueous, and alcoholic (ethanol) extracts of the plants. The phytochemical analysis from the aqueous and ethanolic extract of *A. nilotica* fruits revealed the presence of carbohydrates, reducing sugar, flavonoid, phenol, saponin, steroid, and terpenoids. The results of the phytochemical screening of aqueous and ethanolic extract of *A. nilotica* fruits are given in Table 1. The study revealed the presence of a variety of phytochemicals in *A. nilotica* plant, which makes it a potential source of bioactive components for the drug industry. The phytochemical studies showed the presence of both phenol and flavonoid which are potential natural antioxidant agents to remove free radicals from our body system. The image of fruits and colour developments for the presence of different phytoconstituents in plant extract has been shown in Fig. 1 (a-d).

Table 1: Phytoconstituents in aqueous and ethanol extracts of *Acacia nilotica* fruits.

Phytochemicals	***Acacia nilotica* fruits**	
	Aqueous extract	**Ethanol extract**
Carbohydrates	+++	+++
Flavonoid	+++	+++
Phenol	+++	+++
Reducing sugar	+++	+++
Saponin	+++	-
Steroid	-	++

Tannin	+++	+
Terpenoid	+++	+++

The pharmacological studies revealed that saponins can be used as antibiotic, antifungal, and anti-ulcer agents[31]. Tannins are used as an anti-inflammatory agent and inhibit pathogenic fungi [32].

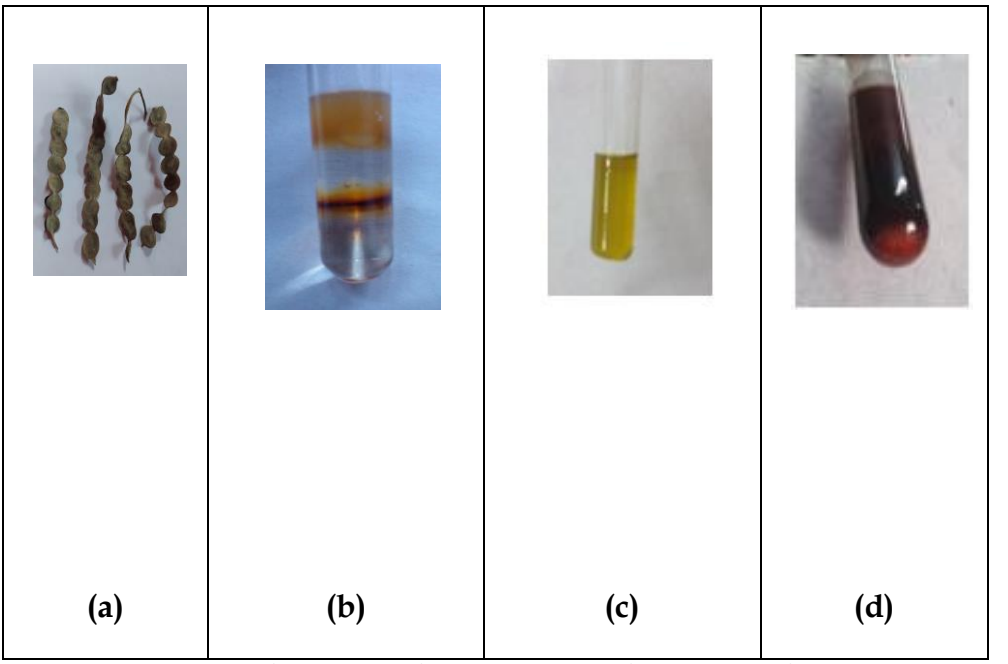

Fig. 1: (a-d): (a) Image of *Acacia nilotica* fruits (b) Salkowski test for the screening of terpenoid in plant extract. (c) Test for flavonoid with the plant extract. (d) Test for reducing sugar in plant extract.

3.2 Quantification of Phenol and Flavonoids

Phenolic compounds like phenol and flavonoid are the most abundant secondary metabolites in the plant kingdom. These compounds act as an antioxidant, and protective agents again UV light. The mechanism of their action is to scavenge free radicals by their hydroxyl groups. Quantitative determinations of individual phenolic compounds are disturbed due to the complexity and diversity of the structure. The spectrophotometric method is useful in solving this problem. The estimation of phenol per ml of plant extract was done by the Folin-Ciocalteu spectrometric method at 550 nm and it was expressed in terms of gallic acid equivalent (mg of GAE/ ml of extract). The contents of phenol for aqueous and

alcoholic (ethanol) extract of *A. nilotica* were3.4±0.027 and 3.6±0.043 mg of GAE/ml of extract respectively. The estimation of flavonoid in per ml plant extract was done by using the aluminum chloride method at 510 nm and it was expressed in terms of quercetin equivalent (mg of QE/ ml of extract. The contents of flavonoid for aqueous and alcoholic (ethanol) extract of *A. nilotica* were8.53±0.027 and 9.18±0.029 mg of QE/ml of extract respectively. These results clearly indicated that solvents influenced the quantity of phenolic compounds from plant extracts.

3.3Antioxidant Activity

Synthetic antioxidants are randomly used in food, cosmetics, and as medicine for their stability, cost-effectiveness, and easy availability. The mostly used synthetic antioxidants are Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and many research studies have reported them as carcinogens in animal studies [33, 34]. In the present study, antioxidant activity was accessed by the Ferric Reducing Antioxidant Power (FRAP) method and the Iron Reducing Power method.

3.3.1 Iron Reducing Power Method

This method is based on the principle of an increase in the absorbance of the reaction mixtures. An increase in the absorbance indicates an increase in antioxidant activity. In this method, the antioxidant compound forms a colour compound with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which is measured at 700 nm. An increase in the absorbance of the reaction mixture indicates the reducing power of the samples[35]. The antioxidant activity is expressed in terms of Ascorbic acid equivalent. The values for Iron Reducing Power (IRP) method were 0.7±0.038 and 0.64±0.033 mg of AA/ml of extract respectively. The high reducing power is indicative of the hydrogen-donating ability of the active species present in the extract.

3.3.2 FRAP Assay

This method measures the ability of antioxidants to reduce ferric ions. It is based on the complex of ferric ion and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to ferrous form at low pH [TPTZ-Fe(III) complex to TPTZ-Fe(II)]. Antioxidant

activity increases linearly with increasing concentration of polyphenols. The estimation is expressed in terms of Ferrous Sulphate equivalent. The results of antioxidant power by using Ferric Reducing Antioxidant Power (FRAP) assay were 10.11±0.035 and 9.75±0.023 mM of $FeSO_4$ respectively.

3.4 Antimicrobial activity

The results (Table 2, Fig. 2) have shown that the alcohol extract of *A. nilotica* displayed amazing antimicrobial activity (IZ: 10.333±0.471mm)against *E. coli*, greater than that of Streptomycin (IZ: 4.333±0.471mm) and slightly greater than that of Tetracycline (IZ: 07±0.816mm). It also showed some considerable antimicrobial activity against *P. aeruginosa* (IZ: 10±0.816mm) and *B. subtilis* (IZ: 10±0.816mm) same and lesser than that of Streptomycin (IZ: 10±0.816mm for *P. aeruginosa*, IZ: 13±0.816mm for *B. subtilis*) but much lesser than that of Tetracycline (IZ: 17.667±1.699mm for *P. aeruginosa*, IZ: 21±0.816mm for *B. subtilis*) (Table 2).

Table 2: Evaluation of the antimicrobial activity of plant extract and standard antibiotics by measuring the zone of inhibition.

Test Microorganisms	**Plant Extracts and Mean Zone of Inhibition (IZ) in mm ± Standard Deviation (SD)**		**Standard Antibiotics (Concentration: 20µg/ml) and Mean Zone of Inhibition (IZ) in mm**	
	Aqueous Extract	**Alcohol Extract**	**Streptomycin**	**Tetracycline**
Pseudomonas aeruginosa	1.667±0.469	10±0.816	10±0.816	17.667±1.699
Staphylococcus aureus	0	09±0.816	13.333±0.471	20.667±0.469
Bacillus subtilis	1.333±0.4714	10±0.816	13±0.816	21±0.816
Escherichia coli	0	10.333±0.471	4.333±0.471	07±0.816

Aqueous extracts of *A.nilotica* showed nominal antimicrobial activity against *P.aeruginosa* (IZ: 1.667±0.469mm) and *B.subtilis* (IZ: 1.333±0.4714mm), and no antimicrobial activity against *S.aureus* and *E.coli* (Fig. 2, 3).

In comparison to these findings, previously *E. coli* and *B.subtilis* havebeen found sensitive to aqueous, ethanolic, and methanolic

extracts of *A.nilotica* [11, 36, 37]. For the future experiments in this perspective and for proper evaluation of the antimicrobial activity of *A.nilotica* plant extracts, minimum inhibitory concentration **(**MIC) by the broth dilution method [38] as well as minimum bactericidal concentration (MBC), should be determined.

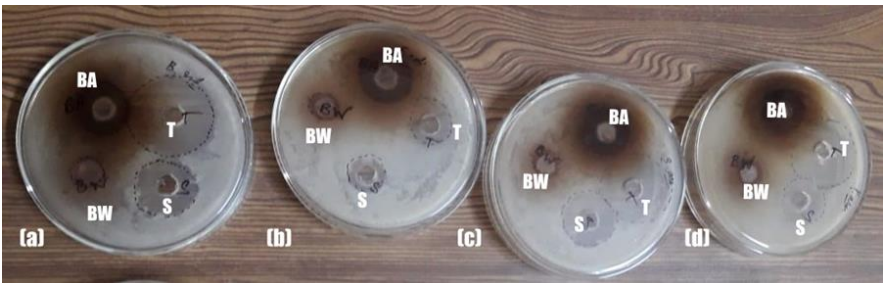

Fig. 2: Antimicrobial activity of *A. nilotica* Fruit extracts (a-d): (a) *B. subtilis*, (b) *E. coli*,(c) *S. aureus*, (d) *P. aeruginosa*. (BW: Aqueous extract of *A. nilotica*, BA: Alcohol extract of *A. nilotica*, S: Streptomycin, T: Tetracycline).

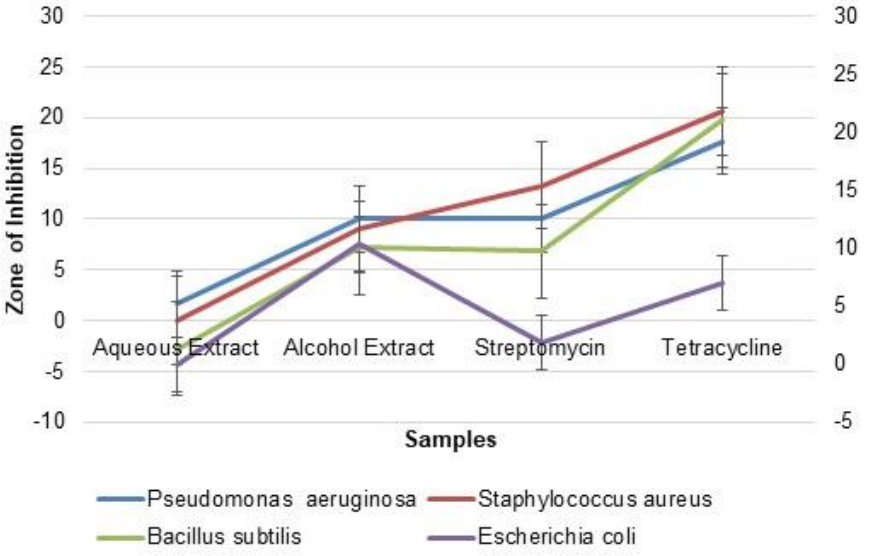

Fig. 3: Depiction of aqueous and alcoholic extract of *Acacia nilotica* fruit extract for antimicrobial activity against standard antibiotics by measuring the zone of inhibition.

3.5 Larvicidal Activity

Aqueous extracts of *Acacia nilotica* fruit were found to show potential toxicity against mosquito larvae. The highest concentration of 10000 mg L^{-1} showed 100% mortality. 100% mortality was also observed in plant extracts as low as 2500 mg L^{-1}. Again, no mortality was

observed for concentrations lower or equal to 10 mg L^{-1} (Fig. 4). Results obtained from the probit analysis revealed the LC_{50} value of 142.074 mg L^{-1}.

Mosquitos are notorious in nature. Apart from creating a considerable amount of annoyance, they spread deadly diseases worldwide [39]. *Aedes albopictus* has been reported as an ardent exophagic and exophilic mosquito having high mammalian affinity [40]. This mosquito species is cosmopolitan in nature and is found all over the world. Being notoriously invasive *Aedes albopictus* is among the major vectors of dengue, Zika viruses, and chikungunya [41, 42]. Controlling these diseases mostly depends on controlling their vector, *Aedes* mosquitoes. Since, the mosquitoes are born in waterbodies, it is the best time to get rid of them at the larval stage. Chemicals used as larvicides have shown success to some extent. However, over time they develop insecticide resistance and *Aedes albopictus* is no exception in this regard [43]. Also, these chemicals are hazardous to nature and may lead to the killing of non-target species as well. Phytochemicals on the other hand are gentle to nature and can be used effectively to control mosquito larvae.

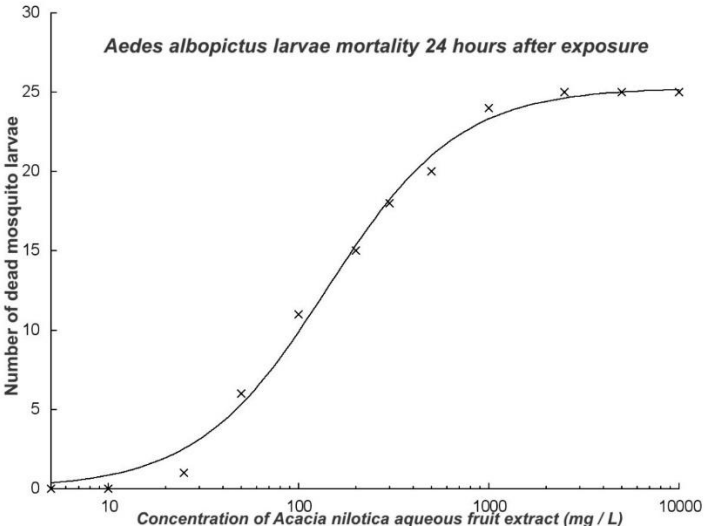

Fig. 4: *Aedes albopictus* mosquito larvae mortality against different concentrations of *Acacia nilotica* aqueous fruit extract.

Previous studies with aqueous extracts from plant leaves against the *Aedes albopictus* mosquito have shown interesting results. Aqueous

extracts of *Syzygiumlanceolatum*leaves have been found to show a LC_{50} value of 72.24 mg L^{-1} [44] while that of *Galinsoga parviflora* leaves have shown LC_{50} value of 49.56 mg L^{-1} [45] against *Aedes albopictus* larvae. In another study aqueous extracts of *Rutachalepensis*leaves have been found to show LC_{50} value of 93.3 mg L^{-1} [46] against *Aedes albopictus* larvae. Aqueous extracts of *Heracleum sprengelianum* leaves have been found to show LC_{50} value of 37.5 mg L^{-1} [47] while that of *Plectranthus barbatus* leaves have shown LC_{50} value of 87.25 mg L^{-1} [48] against *Aedes albopictus* larvae. In another study aqueous extracts of *Ocimumbasilicum* leaves have shown LC_{50} values as low as 11.97 mg L^{-1} [49] against *Aedes albopictus* larvae. Considering the above discussion comment may be made that aqueous extracts of various plants have varied effects on *Aedes albopictus* larvae mortality and hence wide spectrum of LC_{50} values have been reported. As far as *Acacia nilotica* is concerned in a previous study researchers have reported that the raw acetone extract of *Acacia nilotica* had chronic toxicity at 500 mg L^{-1} concentration against *Aedes aegypti* and *Culex quinquefasciatus*4thinstar mosquito larvae [50]. In another study, researchers have reported an estimated LC_{50} value of 0.004% against mosquito larvae for *Acacia nilotica* aqueous fruit extract[51]. Previously it has been reported that *Acacia nilotica* aqueous fruit extract could kill 50% of *Culex* mosquito larvae at 400 mg L^{-1} concentration[52]. The present findings are interesting in this regard where the LC_{50} value of 142.074 mg L^{-1} was found against *Aedes albopictus* larvae using *Acacia nilotica* aqueous fruit extract. The presence of chemical components including flavonoids, saponins, and tannins in the aqueous extracts of *Acacia nilotica* were potent enough to kill *Aedes albopictus* larvae. The present finding corroborates well with that made by other researchers [53]. We plan to use other parts of the same plant on different mosquito species in later days. As of now, it may be stated that *Acacia nilotica* aqueous fruit extract is toxic to *Aedes albopictus* larvae and it can be used to control this mosquito species in an environment-friendly way.

Conclusion

The screening of phytochemicals from medicinal plant sources will help us to understand the presence of different phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides, phenolics, reducing sugars, saponins, steroids, tannins and terpenoids in

various plant parts, including fruits, leaves, sepals, and seeds. These secondary metabolites are responsible for the medicinal properties of these plants and can be used as potential drugs for our health benefits. Quantitative analysis of phenols and flavonoids will help to understand their use as antioxidants. Aquantitative analysis of other phytochemicals should be done to know the number of active constituents present in these plant extracts. The presence of antioxidant activity in *Acacia nilotica* will make it useful as a good natural antioxidant agent for therapeutic purposes. Also, the antimicrobial properties of *Acacia nilotica* may be used for designing suitable drugs. Purifying the active ingredient responsible for mosquito larvae mortality may lead to the inexpensive production of larvicides harmless to non-target species. Also, future studies may be aimed at the application of other extraction methods to monitor mosquito larvicidal activities for *Acacia nilotica* plant species. The present findings are interesting, and mention may be made that the extracts from *Acacia nilotica* plant species can be most effectively used as natural antioxidant, antibacterial, and mosquito larvicidal agents.

References

[1] Ncube, N.S., Afolayan, A.J., Okoh, A.I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, 7, 1797–1806. https://doi.org/10.5897/AJB07.613.

[2] Rios, J.L., Recio, M.C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100, 80–84. https://doi.org/10.1016/j.jep.2005.04.025.

[3] Hérouart, D., Gontier, E., Sangwan, R.S., Sangwan–Norreel, B.S. (1991). Analysis of the potential use of androgenic Datura innoxia for the development of cell cultures producing high amounts of Tropane alkaloids. Journal of Experimental Botany, 42, 1073–1076. https://doi.org/10.1093/jxb/42.8.1073.

[4] Mattson, M.P., Cheng, A. (2006). Neurohormetic phytochemicals: low–dose toxins that induce adaptive neuronal stress responses.

Trends in Neurosciences, 29(11), 632–639. https://doi.org/10.1016/j.tins.2006.09.001.

[5] Singh, U., Jialal, I. (2006). Oxidative stress and atherosclerosis. Pathophysiology, 13, 129–142. https://doi.org/10.1016/j.pathophys.2006.05.002.

[6] Meng, J., Fang, Y., Zhang, A., Chen, S., Xu, T., Ren, Z., Han, G., Liu, J., Li, H., Zhang, Z., Wang, H. (2011). Phenolic content and antioxidant capacity of Chinese raisins produced in Xinjiang Province. Food Research International, 44(9), 2830–2836. https://doi.org/10.1016/j.foodres.2011.06.032.

[7] Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Sharma, B.K., Singh, H.B. (2009a). Antioxidant and anti-quorum sensing activities of green pods of Acacia nilotica L. Food and Chemical Toxicology, 47, 778–786. https://doi.org/10.1016/j.fct.2009.01.009.

[8] Harbottle, H., Thakur, S., Zhao, S., White, D.G. (2006). Genetics of antimicrobial resistance. Animal Biotechnology,17, 111–124. doi: 10.1080/10495390600957092.

[9] Akram, M., Shahid, M., Khan, A.U. (2007). Etiology and antibiotics resistance pattern of community acquired urinary infections in J N M C Hospital Aligarh India. Annals of Clinical Microbiology and Antimicrobials,6, 4. doi: 10.1186/1476–0711–6–4.

[10] Basak, S., Singh, P., Rajurkar, M. (2016). Multidrug resistant and extensively drug resistant bacteria: a study. 2016, 1–5.Journal of Pathogens, http://dx.doi.org/10.1155/2016/4065603.

[11] Dabur, R., Gupta, A., Mandal, T.K., (2007). Antimicrobial activity of some medicinal plants. African Journal of Traditional, Complementary and Alternative Medicines, 4(3), 313–318. http:// doi.org/10.4314/ajtcam.v4i3.31225.

[12] World Health Organization. 2020. Urgent health challenges for the next decade. https://www.who.int/news-room/photo-story/photo-story-detail/urgent-health-challenges-for-the-next-decade.

[13] Vatandoost, H., Vaziri, M. (2001). Larvicidal activity of neem extract (Azadirachta indica) against mosquito larvae in Iran. Pestology, 25, 69–72.

[14] Das, M.K., Ansari, M.A. (2003). Evaluation of repellent action of Cymbopoganmartiniimartinii. Stapf var sofia oil against Anopheles sundaicus in tribal villages of Car Nicobar Island, Andaman Nicobar Islands, India. Journal of Vector Borne Diseases, 40, 100–104. http://www.mrcindia.org/journal/issues/403100.pdf.

[15] Nigam, S.K., Venkatakrishna, B.H., Bhatt, H. (2001). Occupational cancer: Introduction and intervention. Indian Journal of Occupational Health, 44, 79–88.

[16] Mahmood, I., Imadi, S.R., Shazadi, K., Gul, A., Hakeem, K.R. (2016). Effects of pesticides on environment. In: Hakeem, K., Akhtar, M., Abdullah, S. (eds) Plant, soil and microbes. Springer, Cham, pp 253–269. https://doi.org/10.1007/978-3-319-27455-3_13.

[17] Aktar, M.W., Sengupta, D., Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology, 2(1), 1–12. https://doi.org/10.2478/v10102-009-0001-7.

[18] Chowdhury, N., Laskar, S., Chandra, G. (2008). Mosquito larvicidal and antimicrobial activity of protein of Solanum villosum leaves. BMC Complementary and Alternative Medicine, 8, 62. https://doi.org/10.1186/1472-6882-8-62.

[19] Rawani, A., Ghosh, A., Chandra, G. (2013). Mosquito larvicidal and antimicrobial activity of synthesized nano–crystalline silver particles using leaves and green berry extract of Solanum

nigrum L. (Solanaceae: Solanales). Acta Tropica, 128(3), 613–22. https://doi.org/10.1016/j.actatropica.2013.09.007.

[20] Rather, L.J., ul-Islam, S., Mohammad, F. (2015). Acacia nilotica (L.): A review of its traditional uses, phytochemistry, and pharmacology. Sustainable Chemistry and Pharmacy, 2, 12-30. http://dx.doi.org/10.1016/j.scp.2015.08.002i.

[21] Gupta, R.S., Kachhawa, J.B., Chaudhary, R. (2004). Antifertility effects of methanolic pod extract of Albizia lebbeck Benth. in male rats. Asian Journal of Andrology, 6(2), 155–159.

[22] Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa (pp. 150–153). John Wiley and son Ltd.

[23] Evans, W.C. (2002). Trease and Evans Pharmacogonasy, 15th ed., W.B. Sounders Co., London.

[24] Idu, M., Igeleke, C.L. (2012). Antimicrobial activity and Phytochemistry of khaya senegalensis roots. International Journal of Ayurvedic and Herbal Medicine, 2, 415– 422. https://doi.org/10.17582/journal.pjar/2019/32.1.46.51.

[25] Singleton, V.L., Orthofer, R., Lamuela–Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymology, 299, 152–179. https://doi.org/10.1016/S0076–6879(99)99017–1.

[26] Ghasemzadeh, A., Hawa, Z.E., Jaafar, A.R. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia Young Ginger (Zingiber officinale Roscoe). Molecule, 15, 4324–4333. https://doi.org/10.3390/molecules15064324.

[27] Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complimentary colorimetric methods. Journal of Food and Drug

Analysis, 10, 178–182. https://doi.org/10.38212/2224–6614.2748.

[28] Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of product of browning reaction prepared from glucosamine. Japan Journal of Nutrition, 44, 307–315. https//dx.doi.org/10.5264/eiyogakuzashi.44.307.

[29] Benzie, I.F.F., Strain, J.J. (1999). Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymology, 299, 15–27. https://doi.org/10.1016/s0076–6879(99)99005–5.

[30] World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides. https://apps.who.int/iris/handle/10665/69101.

[31] Zhang, Y.W., Due, D.Q., Zhang, L., Chen, Y.Z., Yao, X.S. (2001). Effects of Ginsenosides from Panax ginseng on cell–to–cell communication function mediated by gap junctions, Planta Medica, 67, 417–422. https://doi.org/10.1055/s–2001–15816.

[32] Lalitha, T.P., Jayanti, P. (2012). Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms. Asian Journal of Plant Science and Research, 2, 115–122.

[33] Saad, B., Sing, Y.Y., Nawi, M.A., Hashim, N., Mohamedali, A., Saleh, M.I., Sulaiman, S.F., Talib, K., Ahmad, K., Ali, A.S.M. (2007). Determination of synthetic phenolic antioxidants in food items using reversed–phase HPLC. Food Chemistry, 105, 389–394. https://doi.org/10.1016/j.foodchem.2006.12.025.

[34] Botterweck, A., Verhagen, H., Goldbohm, R., Kleinjans, J., Brandt, P.V.D., Brandt, P.V.D. (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort

Study. Food and Chemical Toxicology, 38, 599–605. https://doi.org/ 10.1016/S0278–6915(00)00042–9.

[35] Olayinka, A.A., Anthony, I.O. (2010). Preliminary phytochemical screening and In vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complementary and Alternative Medicine, 10(21), 1–8. https://doi.org/10.1186/1472–6882–10–21.

[36] Kaur, G., Sharma, A.K., Karnwal, A. (2016). Antimicrobial activity of Acacia nilotica against various clinical isolates. Elixir Applied Botany, 97, 42260–42262.

[37] Arias, M.E., Gomez, J.D., Cudmani, N.M., Vattuone, M.A., Isla, M.I. (2004). Antibacterial activity of ethanolic and aqueous extracts of Acacia aroma Gill. ex Hook et Arn. Life Sciences, 75(2), 191–202. https:// doi.org/10.1016/j.lfs.2003.12.007.

[38] Iwaki, K., Koya–Miyata, S., Kohno, K., Ushio, S., Fukuda, S. (2006). Antimicrobial activity of Polygonum tinctorium Lour: extract against oral pathogenic bacteria. Journal of Natural Medicine, 60, 121–125. https://doi.org/10.1007/s11418–005–0025–z.

[39] Barik, M., Rawani, A., Laskar, S., Chandra, G. (2019). Evaluation of mosquito larvicidal activity of fruit extracts of Acacia auriculiformis against the Japanese encephalitis vector Culex vishnui. Natural Product Research, 33(11), 1682–1686. https://doi.org/10.1080/14786419.2018.1428585.

[40] Hawley, W.A. (1988). The biology of Aedes albopictus. Journal of the American Mosquito Control Association. Supplement, 1, 1–39.

[41] Gratz, N.G. (2004). Critical review of the vector status of Aedes albopictus. Medical and veterinary entomology, 18, 215–227. https://doi.org/10.1111/j.0269-283X.2004.00513.x.

[42] Paupy, C., Delatte, H., Bagny, L., Corbel, V., Fontenille, D. (2009). Aedes albopictus, an arbovirus vector: from the darkness to the light. Microbes and infection, 11, 1177–1185. https://doi.org/10.1016/j.micinf.2009.05.005.

[43] Bharati, Minu., Rai, P., Saha, D. (2019). Insecticide resistance in Aedes albopictus Skuse from sub–Himalayan districts of West Bengal, India. Acta Tropica, 192, 104–111. https://doi.org/10.1016/j.actatropica.2019.02.007.

[44] Benelli, G., Rajeswary, M., Govindarajan, M. (2018). Towards green oviposition deterrents? Effectiveness of Syzygiumlanceolatum (Myrtaceae) essential oil against six mosquito vectors and impact on four aquatic biological control agents. Environmental science and pollution research international, 25(11), 10218–10227. https://doi.org/10.1007/s11356-016-8146-3.

[45] Govindarajan, M., Vaseeharan, B., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Al–Anbr, M.N., Alyahya, S.A., Maggi, F., Benelli, G. (2018). High efficacy of (Z)–γ–bisabolene from the essential oil of Galinsoga parviflora (Asteraceae) as larvicide and oviposition deterrent against six mosquito vectors. Environmental science and pollution research international, 25(11), 10555–10566. https://doi.org/10.1007/s11356-018-1203-3.

[46] Bedini, S., Flamini, G., Ascrizzi, R., Venturi, F., Ferroni, G., Bader, A., Girardi, J., Conti, B. (2018). Essential oils sensory quality and their bioactivity against the mosquito Aedes albopictus. Scientific reports, https://doi.org/10.1038/s41598-018-36158-w.

[47] Govindarajan, M., Benelli, G. (2016). Eco-friendly larvicides from Indian plants: effectiveness of lavandulyl acetate and bicyclogermacrene on malaria, dengue and Japanese encephalitis mosquito vectors. Ecotoxicology and environmental safety., 133, 395–402. https://doi.org/10.1016/j.ecoenv.2016.07.035.

[48] Govindarajan, M., Rajeswary, M., Hoti, S.L., Bhattacharyya, A., Benelli, G. 2016. Eugenol, α–pinene and β–caryophyllene from Plectranthus barbatus essential oil as eco–friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. Parasitology research, 115(2), 807–815. https://doi.org/10.1007/s00436-015-4809-0.

[49] Govindarajan, M., Sivakumar, R., Rajeswary, M., Yogalakshmi, K. (2013). Chemical composition and larvicidal activity of essential oil from Ocimumbasilicum (L.) against Culex tritaeniorhynchus, Aedes albopictus and Anopheles subpictus (Diptera: Culicidae). Experimental parasitology, 134(1), 7–11. https://doi.org/10.1016/j.exppara.2013.01.018.

[50] Chaubal, R., Pawar, P.V., Hebbalkar, G.D., Tungikar, V.B., Puranik, V.G., Deshpande, V.H., Deshpande, N.R. (2005). Larvicidal activity of Acacia nilotica extracts and isolation of D–pinitol—a bioactive carbohydrate. Chemistry & biodiversity, 2(5), 684–8. https://doi.org/10.1002/cbdv.200590044.

[51] Elkhidr, M.E., Abdo, I., Madani, M., Abdelghani, S., Waggiallah, H.A., Eltayeb, L.B. (2020). Toxicity of water extract of Acacia nilotica fruits against mosquito larvae: an experimental study. Entomology and Applied Science Letters, 7(3), 84–90.

[52] Taura, D.W., Mukhtar, M.D., Adoum, O.A. (2004). Lethality of the aqeous extracts of Acacia nilotica, Guiera senegalensis, Kigeliaafricana and Securidacalongepedunculata on culex mosquito larva. Ife Journal of Science, 6(2), 115–118. https://doi.org/10.4314/ijs.v6i2.32135.

[53] Musau, J.K., Mbaria, J.M., Nguta, J.M., Mathiu, M., Kiama, S.G. (2016). Phytochemical composition and larvicidal properties of plants used for mosquito control in Kwale County, Kenya. International Journal of Mosquito Research, 3(3), 12–17.

Author contribution statement:

Dr. Niloy Das has carried out all the laboratory works pertaining to screening and quantification of phytochemicals and antioxidant assay during the present study. He has also written parts of the manuscript related to screening and quantification of phytochemicals and antioxidant assay.

Sonali Saha has carried out antimicrobial studies and has also written parts of the manuscript related to antimicrobial studies.

Dr. Utpal Singha Roy has carried out larvicidal bioassays and has also written part of the manuscript related to mosquito larvicidal studies. He has also made the final composition and acted as corresponding author.

Conflict of interest

The authors would like to declare no competing financial interest for the present manuscript.

Acknowledgements

The authors would like to thank Sri Swapan Sarkar, Officer-in-Charge, P. R. Thakur Government College for his sustained goodwill and encouragement. We further extent our gratitude to Dr. Prasenjit Das (Head, the Microbiology Department, Vijaygarh Jyotish Ray College), for providing bacterial samples. Authors thankfully acknowledge DSTBT, Government of West Bengal for providing necessary funding to carry out the present research work.

Funding information

Minor Research Project sanctioned by DSTBT, Government of West Bengal, [vide sanction no. 1949(Sanc.)/ST/P/S&T/15G-14/2019; dated 26.02.2020].