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EVALUATION OF ANTIOXIDANT AND LARVICIDAL ACTIVITIES OF SELECTED TAMARIX SPECIES AGAINST THE SOUTHERN HOUSE MOSQUITO "*CULEX QUINQUEFASCIATUS* (SAY)"

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Abstract

The present study was aimed to evaluate *Tamarix baluchistanica* (*Qaiser), Tamarix androssowii* (Bunge) and *Tamarix mascatensis* (Bunge) for scavenging of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS) free radicals and larvicidal potential against mosquito *Culex quinquefasciatus*; an important vector for lymphatic filariasis. The aerial parts of plants dried in shade, extracted with methanol and subsequently evaluated for antioxidant and larvicidal activities. The Crude extracts of *T. baluchistanica, T. androssowii* and *T. mascatensis* exhibited ABTS scavenging of 17%, 4.6% and 6% at concentration of 20 µg/ml and 50%, 23% and 30% at concentration of 100 µg/ml, respectively, similar results were observed for DPPH free radicals scavenging activity. The most active methanolic extract of *T. baluchistanica* was subjected to fractionation. Antioxidant bioassays revealed that ethyl acetate fraction was most potent among all the fractions, exhibited IC_{50} of 32.46 µg/ml and IC_{90} of 91.32 µg/ml against DPPH radicals. This was similar to the standard antioxidant, ascorbic acid which showed IC_{50} of 32.48 µg/ml and IC_{90} of 86.73 µg/ml. Antioxidant activity against ABTS demonstrated that among all the fractions, least IC_{50} of 37.94 µg/ml and IC_{90} of 129.85 µg/ml was also recorded for ethyl acetate fraction of *T. baluchistanica* extract, comparable to reference antioxidant, BHT having IC_{50} of 36.78 µg/ml and IC_{90} of 113.07 µg/ml. The crude extract of *T. baluchistanica* showed more larvicidal activity against the 3rd instar larvae of *Cx. quinquefasciatus*. The chloroform fractions showed lowest LC_{50} of 0.06 mg/ml, followed by *n*-hexane fraction having LC_{50} of 1.26 mg/ml, while lowest LC_{90} (2.12 mg/ml) was exhibited by *n*-hexane fraction, followed by chloroform fraction (7.94 mg/ml). The antioxidant and larvicidal activity of these species are reported for the first time. Further research is required to isolate and identify the active components of the plant extracts for effective implication in the control of the vector.

Key words: *Tamarix baluchistanica*, Antioxidant, DPPH, *Culex quinquefasciatus*, Mosquito.

Introduction

Due to scarcity of medical facilities in the rural arears of developing countries including Pakistan, there are a lot of medicinal plants based traditional remedies that are used for treatment of various aliments like bacterial and fungal infections, diarrhea, hepatitis, skin infections, fever, malaria, diabetes and respiratory problems (Afzal *et al.,* 2013; Hamza *et al.*, 2020). A great number of medicinally important plants have been screened for their therapeutic potential against various important diseases, however a wide variety of the indigenous medicinal plants are still not tested according standard protocols (Mahesh & Satish, 2018). Plants that exhibits positive or negative pharmacological effects on the human and animal physiology are often included in medicinal plants. These plants contain phytochemicals that are secondary metabolites of plants i.e. phenols, alkaloids, glycosides, flavonoids, lactones, terpenes, volatile oil compounds, saponins, tannins, coumarins etc. These compounds are biologically active, exerts a variety of health benefits in human beings (Ullah *et al*., 2017). The phytochemicals are mainly meant for fortification and defense of plants, but recent researches have established their efficacy to protect humans and animals against various diseases (Phillipson, 1999). The medicinal plants that demonstrates high antioxidant activities attract many researchers to consider them for more studies for the treatment of diverse type of diseases such as hyperglycemia, hypertension, cancer, atherosclerosis, hepatitis, renal and cardiovascular problems (Vaghasiya *et al*., 2011; Rebaya *et al.*, 2015). Due to increase in demand of safe and non-hazardous alternative antioxidants, the analysis of phytochemicals for their antioxidant activities has been highly increased recently (Aliyu *et al*., 2013). Mosquito is carrier of pathogens responsible for spread of numerous stern human ailments e.g. malaria, yellow fever, dengue fever, chikungunya, Japanese encephalitis, and lymphatic filariasis etc. (Chowell *et al*., 2011). As mosquito plays a vital role in spread of many important diseases, its control is as essential task for the world community to avoid certain lethal disease and unbearable biting irritations (Curtis, 1994; Collins *et al.*, 1995 and Gubler *et al.*, 1998). Synthetic pesticides have been extensively used for control of mosquitoes, has developed resistant insect strains, that caused ecological imbalance resulted in treats to animals and human (Georghiou & Lagunes-tejeda, 1991). The best strategy to avoid ecological problems could thus be eliminated using plant-based alternative insecticides that are more easily degradable, ecofriendly and their source is renewable (Roel, 2001). *Cx. quinquefasciatus* the chief vector "lymphatic filariasis" that is commonly found in tropical regions (Bernhard *et al.*, 2003).

The genus "Tamarix" belongs to family Tamaricaceae. The plants are traditionally used as ethno-medicine for treatment of several ailments e.g. diabetes, febrifuge, dermatosis and paralysis of upper limb (Bhadange & Jadhao, 2013).The decoction of *Tamarix aphylla* (L.) can be curative for internal wounds in body (Naz *et al*., 2014).

Earlier reports of Saidana *et al*., (2007), established that chloroform and ethyl acetate extracts of *Tamarix boveana* demonstrated strong anti-feedant and anti-larval activity against *Tribolium confusum*. Soummane *et al*., (2011) described that methanolic extract of *Tamarix gallica* exhibited substantial larvae killing of *Ceratitus capitata*. Researchers suggested the antioxidant and larvicidal assessment of *Tamarix* spp. (Saidana *et al*., 2007; Rahuman *et al*., 2008; Koche *et al*., 2010; Soummane *et al*., 2011; Bhadange & Jadhao, 2013; Naz *et al*., 2014). Based on the remarkable larvicidal action of *Tamarix* spp. against further researches are required to evaluate in depth potential insects (Saïdana *et al.*, 2007; Soummane *et al*., 2011).

Due to the ecofriendly nature, cost-effectiveness and specificity in action, the extracts of Tamarix plants can be employed as natural control agent. Literature review reveled that there are no reports on the antioxidant and mosquitocidal action of selected species of genus Tamarix. Therefore, the present research activity was aimed to evaluate the antioxidant and larvicidal potential of methanolic extract of aerial parts of *T. baluchistanica, T. androssowii* and *T. mascatensis* and their subsequent fractions, through activity guided bioassays.

Materials and Methods

Chemicals: DPPH (2, 2-diphenyl-1-picryl-hydrazyl), Ascorbic acid (As-A), BHT (butylated hydroxytoluene), ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonate) and sodium carbonate (Na2CO3) were purchased from Sigma Co. (USA). Analytical grade methanol, n-hexane, ethyl acetate, chloroform and butanol used for plant extraction and fractionation were obtained from Merck Co. (Darmstadt, Germany).

Collection of plant material: The shoot parts of the three selected plants; *T. baluchistanica, T. androssowii* and *T. mascatensis* were collected each at their flowering stage form Ziarat Balochistan (2554.2 m), Quetta Balochistan (1694.6 m) and Hunza valley Gilgit-Baltistan (2525.8 m) respectively, at flowering stage. The specimens were identified by Dr. Prof. Mir Ajab Khan, National Herbarium, Department of Plant Sciences Quaid-i-Azam University Islamabad, Pakistan.

Extraction and fractionation: The plant material (aerial shoots including leaves and flowers) was cleaned properly, by washing with distilled water, dried in shade and coarsely ground with a mechanical grinder. The powdered plant material (10 Kg) was extracted with 80% methanol at 25°C for 15 days at room temperature with occasional shaking. After 5 days, the soaked material was filtered with Whatman No.6 filter paper and stored in refrigerator at 4°C. The process was repeated three times and the combined filtrate of each plant was evaporated by using rotary evaporator (Heidolph Laborta 4000 efficient Germany) till a reddish-brown gummy crude methanolic extract was obtained yielding 670g extract of *T. baluchistanica,* 484g extract of *T. androssowii* and 325g extract of *T. mascatensis*. In the next step, each extract was then dissolved in distilled water (3ml/gm) of extract and subsequently fractioned with equal volume of *n*-hexane, chloroform, ethyl acetate and butanol. The process of fractionation was repeated three times using the solvent in increasing order of polarity. The residue left behind in each process was treated as aqueous fraction and were then stored separately 4°C in refrigerator.

Sampling of Mosquito: The mosquitoes (*Cx. quinquefasciatus*) were collected from the ditches near University of Malakand, Dir, KPK. These mosquitoes were made half anesthetic and then identified for the breeding purpose. They were taken for breeding (Ilahi *et al.*, 2012).

Raring of larvae: Adult mosquitoes *Cx. quinquefasciatus* were kept in controlled laboratory for breeding in the enclosed mosquitoes net. The mosquitoes were allowed, to bite and suck blood from rats. The mosquitoes laid eggs in tray containing tap water (culture medium) under laboratory condition (30°C). After 24 h incubation, the eggs were observed to hatch out into first instar larvae. An appropriate amount of nutrients such as; sterilized yeast powder and dog biscuit in 1:1 ratio, (at the rate of 1gm/100 ml of water) were added for growth of larvae. After 2 days the larvae were observed as 3rd instar. These larvae were used in the study (Ilahi *et al.*, 2012).

Larvicidal bioassay: DMSO (5 drops) was added to the extract. Extract (1g) of each plant and its fraction were dissolved in 100 ml of water. The resultant stock solution at concentration of 10mg/ml was used, from which dilutions were made as, 5mg/ml, 2.5 mg/ml and 1.25 mg/ml. The plant extracts were tested at all the concentrations for the larvicidal activity. The experiment was repeated three times. A corresponding negative control was maintained for the authentication of the activity. The larval mortality of 3rd instar of *Cx. quinquefasciatus* was observed after 24 hours of the incubation period. The number of larvae killed after 24 hours were recorded, and the percent mortality was calculated by using the formula;

$$\text{Mortility (\%)} = \frac{\text{Number of larve killed}}{\text{Total number of larvae}} \text{ X } 100$$

Antioxidant activity

DPPH free radical scavenging assay: DPPH radical scavenging potential of the extracts and fractions was carried out by the method described by Huang *et al.*, (2010). 1 ml of the extract solution having concentration of 20, 40, 60, 80 and 100 µg/ml in ethanol was poured into different test-tubes. 1ml of DPPH solution of concentration 0.2mM in ethanol was then added to each test-tube. The solution was kept for 30 minutes to react at room temperature. Absorbance of each solution was checked with UV-light by using spectrophotometer at 517 nm. Ascorbic acid was used as standard antioxidant for the activity while, blank solutions of only DPPH in ethanol were used as negative control for comparison. Percent DPPH free radical quenching was determined by using the formula: The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavinging(\%)} = \frac{\text{A0} - \text{A1}}{\text{A0}} \text{ X } 100$$

where, A0 is the absorbance of control and A1 is the absorbance of standard.

ABTS cation scavenging activity: The ABTS free radical scavenging potential of the extracts was evaluated by the method of Huang *et al.*, (2011). ABTS cation radical was induced by reacting 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution. The reaction solution was stored in dark at room temperature for 16 hours. The solution was then diluted with ethanol to obtain absorbance of 0.7 ± 0.02 at 734 nm. The plant extract (1 ml) at various concentrations; 20, 40, 60, 80 and 100 µg/ml in ethanol was added into separate test-tubes and 1ml of ABTS solution was poured and then homogenized. Similar concentrations of solutions were made for the BHT. The absorbance was recorded after 6 minutes of incubation, at 734 nm. Ethanol blanks were run in each test for calculation of radical reducing. The ABTS scavenging capacity was expressed as IC_{50} and IC_{90} (µg/ml) by analyzing data through SPSS 16.0 statistics software. The percent inhibition of ABTS radical was measured, using the following formula:

$$\text{ABTS scavinging}(\%) = \frac{\text{A0} - \text{A1}}{\text{A0}} \text{ X } 100$$

where, A0 is the absorbance of control and A1 is the absorbance of standard.

Statistical analysis

The experiment was designed according to CRD model. The data was subjected to Tukey HSD and analyzed by One-way ANOVA using software Statistix 8.1.The data was analyzed by Probit analysis via SPSS 16.0, the values for antioxidant activity were expressed as IC_{50} and IC_{90} while, in case of larvicidal activity the values were expressed as LC_{50} and LC_{90}.

Results and Discussion

The DPPH is a stable free radical that is extensively used to investigate the antioxidant efficacy of plant extracts (Onyeulo *et al.,* 2018), have maximum absorption at 517 nm. The methanolic crude extracts of *T. baluchistanica* (TB-Cr), *T. androssowii* (TA-Cr) and *T. mascatensis* (TM-Cr) were analyzed for free radical scavenging of DPPH free radicals. All the plants demonstrated variable DPPH free radical quenching potential at different concentration as shown in Fig. 1. The activity followed a concentration dependent trend in the current study, where TB-Cr exhibited significant scavenging of DPPH; 21.6% and 44.6% at the lowest 20 µg/ml and highest 100 µg/ml concentration, respectively at $p<0.05$. The Crude methanolic extracts of *T. androssowii* and *T. mascatensis* exhibited DPPH scavenging activity of 6% and 13% at lowest concentration of 20 µg/ml and 23% and 33% at the highest concentration of 100 µg/ml, respectively. Further, the potential of various fractions of TB-Cr was established as given in Fig. 2. These results strongly endorse the work of Naz *et al.*, (2014) that demonstrated significant antioxidant activities of Tamarix spp. The ethyl acetate fraction (EtAc) of TB-Cr showed significant DPPH activity of 40.3% at lowest 20 µg/ml and 93.3% highest 100 µg/ml at as compared to all the other fractions of TB-Cr at $p<0.05$, whereas the rank of order was uniform at all the concentrations; As-A > EtAc > but > TB-Cr > n-Hex > Chl > Aqu. The standard antioxidant Ascorbic acid (As-A), showed significantly higher activity of scavenging DPPH free radicals; 44.3% at 20 µg/ml and 98.6% at 100 µg/ml as compared to all the fractions at $p<0.05$. The IC_{50} and IC_{90} values DPPH scavenging of all the fractions and As-A expressed in µg/ml, are given in (Table 1). Among all the treatment groups minimum IC_{50} of 32.46 µg/ml and IC_{90} of 91.32 µg/ml were recorded for EtAc, while similar IC_{50} of 32.48 µg/ml and IC_{90} of 86.73 µg/ml was found for As-A. All the other treatment groups showed higher IC_{50} and IC_{90} values for the DPPH inhibition activity. Our current results strongly accords to the work of Bakr *et al*., (2013), who established that the DPPH free radical scavenging assay of different *Tamarix nilotica* fractions i.e. Ethyl acetate (100%), Butanol (93%) and crude extract (90%) at 100 µg/ml, exhibited potential antioxidant action, while Chloroform fraction exhibited the lowest effect (26%). The IC_{50} of promising ethyl acetate fraction (>90%) when compared with standard ascorbic acid (IC_{50} 4.8 ± 0.54 µg/ml), ethyl acetate fraction showed the best effect (7.25 ± 0.86 µg/ ml), with lower IC_{50} followed by butanol fraction (8.25 ± 0.65µg/ml) and total extract (45 ± 0.73µg/ml).

Table 1. IC_{50} and IC_{90}(µg/ml) of methanolic plant extracts of aerial parts of *T. baluchistanica T. Androssowii* and *T. mascatensis* for DPPH and ABTS free radical scavenging activity.

Groups	DPPH		ABTS	
	IC_{50} µg/ml	IC_{90} µg/ml	IC_{50} µg/ml	IC_{90} µg/ml
Methanolic Extract	77.63	193.19	99.22	204.45
n-hexane fraction	160.82	331.85	165.3	289.38
Chloroform fraction	131.89	293.14	149.47	274.08
Ethyl acetate fraction	32.46	91.32	37.94	129.85
Butanol fraction	49.1	139.43	49.95	175.33
Aqueous fraction	185.57	356.59	164.22	251.32
Standard	32.48	86.73	36.78	113.07

Standards; DPPH inhibition: ascorbic acid, ABTS inhibition: BHT

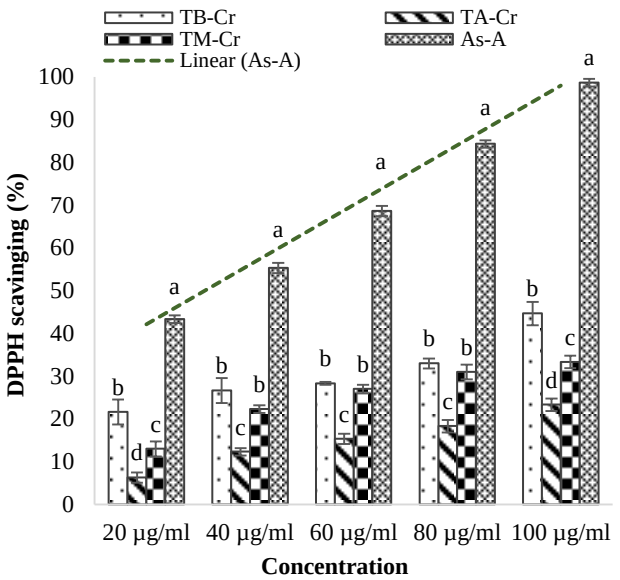

Fig. 1. Percent DPPH scavenging activity of methanolic crude extracts of aerial parts of *T. baluchistanica, T. androssowii* and *T. mascatensis*. All values are mean (± standard error) of three replicates where similar letters are not significantly different at $p<0.05$. Whereas; TB: *T. baluchistanica*, TA: *T. androssowii*, TM: *T. mascatensis*, Cr: Crude extract, As-A: ascorbic acid.

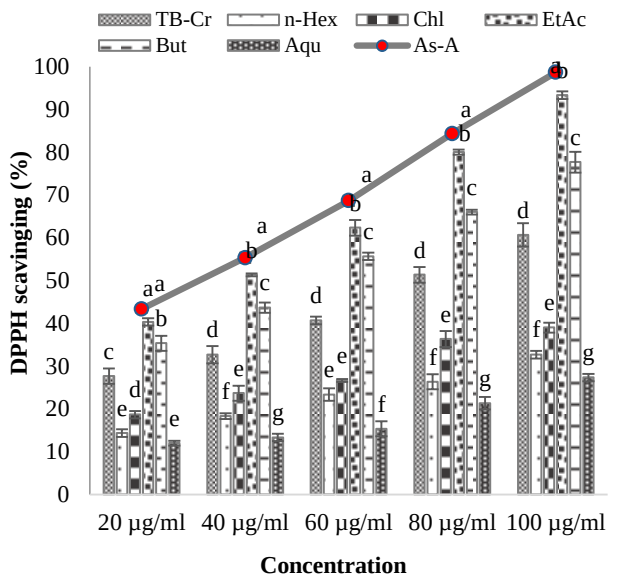

Fig. 2. Percent DPPH free radical scavenging of methanolic extract and their fractions of *T. baluchistanica* (aerial parts). All values are mean (± standard error) of three replicates where similar letters are not significantly different at $p<0.05$.TB-Cr: *T. baluchistanica* methanolic extract; *n*-Hex: *n*-hexane fraction; Chl: chloroform fraction; EtAc: ethyl acetate fraction; But: butanol fraction; Aqu: aqueous fraction; As-A: ascorbic acid.

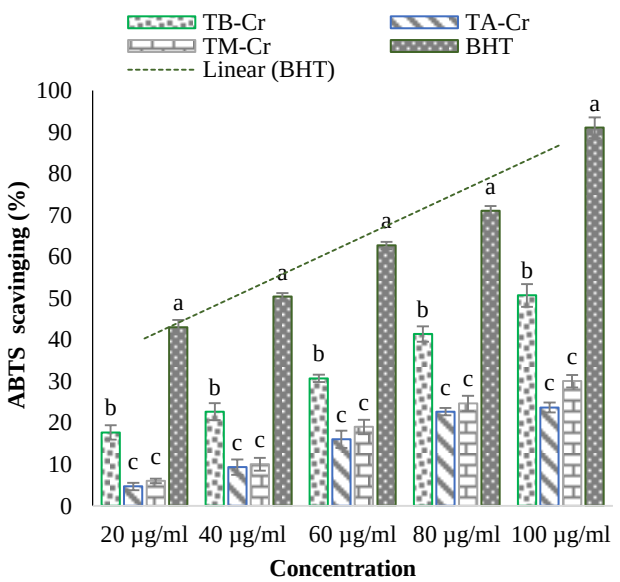

Fig. 3. ABTS cation scavenging activity of methanolic crude extracts of aerial parts of *T. baluchistanica, T. androssowii* and *T. mascatensis* against at $p<0.05$. Whereas; TB: *T. baluchistanica*, TA: *T. androssowii*, TM: *T. mascatensis*, Cr: Crude extract, BHT: butylated hydroxylated toluene.

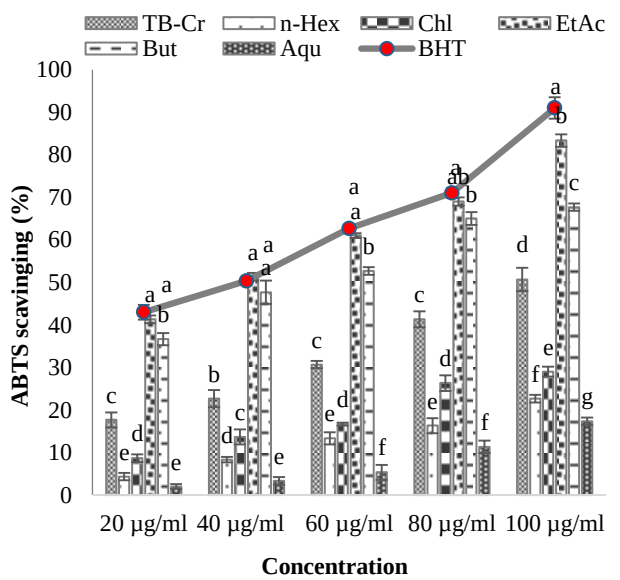

Fig. 4. Percent DPPH free radical scavenging of methanolic extract and their fractions of *T. baluchistanica* (aerial parts). All values are mean (± standard error) of three replicates where similar letters are not significantly different at $p<0.05$.TB-Cr: *T. baluchistanica* methanolic extract; *n*-Hex: *n*-Hexane fraction; Chl: chloroform fraction; EtAc: ethyl acetate fraction; But: butanol fraction; Aqu: aqueous fraction; BHT: butylated hydroxylated toluene.

The ABTS radical scavenging assay employs to specific absorbance at 734 nm wavelength (Huang *et al.*, 2011) that demonstrates the antioxidant action of the tested samples. In Fig. 3, TB-Cr showed significantly higher activity as compared to the other plant extracts at $p<0.05$. At highest concentration of 100 µg/ml it caused 50.6% inhibition of the ABTS free radicals. Lower IC_{50} (99.22 µg/ml) and IC_{90} (204.45 µg/ml) was recorded for TB-Cr (Table 1).Concentration dependent increase in activity was observed for all plant extracts and BHT. Further study established that among various fractions of TB-Cr the EtAc fraction showed significant inhibition of ABTS; 41.3% at lowest 20 µg/ml and 81.3% highest 100 µg/ml as compared to all the other fractions and TB-Cr at $p<0.05$ (Fig. 4). The rank of order followed same regime as that of DPPH inhibition. The standard BHT, showed significantly higher inhibition of

ABTS cations; 43 % at 20 µg/ml and 91% at 100 µg/ml as compared to all the fractions at $p<0.05$. The IC_{50} and IC_{90} values for ABTS cation inhibition are given in Table 1. Among all the plant extracts/fractions minimum IC_{50} of 37.94 µg/ml and IC_{90} of 129.85 µg/ml were noted for EtAc, while parallel IC_{50} of 36.78 µg/ml and IC_{90} of 113.07 µg/ml was also found for BHT. All the other treatment groups showed higher IC_{50} and IC_{90} values for the ABTS cation inhibition. These results are in accordance to the previous reports of Rahuman *et al*., (2008), Koche *et al*., (2010) and Soummane *et al*., (2011), who showed remarkable antioxidant activity of the organic solvent extracts of other species of genus Tamarix.

Table 2. LC_{50} and LC_{90} of different solvent fractions of methanolic plant extract of *T. baluchistanica* against, 3rd instar larvae of *Cx. quinquefasciatus*, after 24 h of exposure.

Groups	Larvicidal activity	
	LC_{50} mg/ml	LC_{90} mg/ml
Methanolic Extract	0.76	11.7
n-hexane fraction	1.26	2.12
Chloroform fraction	0.06	7.94
Ethyl acetate fraction	19.46	41.54
Butanol fraction	33.55	83.61
Aqueous fraction	19.8	42.5

Larvicidal activity: The results of the larvicidal activity of all the plants methanolic crude extracts i.e. TB-Cr, TA-Cr and TM-Cr are presented in Fig. 5. Linear increase in mortality rate of the mosquito larvae was observed with increase in concentration was observed for all extracts**.** Significantly higher killing mosquito larvae of *Cx. quinquefasciatus* i.e. 45.3% at minimum dose of 1.25 mg/ml and 83.6% at maximum dose of 10 mg/ml, was caused by TB-Cr as compared to other extracts, after 24 hours of incubation ($p<0.05$). Earlier report of Soummane *et al.*, (2011) supports our results, as described that methanolic extract of *T. gallica* revealed significant larvae killing of *C. capitata*. Further investigation of fractions of TB-Cr reveled that significantly higher larval mortality of 49%, 96.6%, 100%, 100% at concentration of 1.25, 2.5, 5, 10 mg/ml, respectively was due to *n*-Hex fraction as compared to all the other fractions at $p<0.05$ (Fig. 6). The rank of order of the larvicidal activity was such that maximum mortality was caused by n-Hex > Chl > TB-Cr > but > EtAc = Aqu. The LC_{50} and LC_{90} for all the fractions of TB-Cr are given in (Table 2). Lowest LC_{50} (0.06 mg/ml) was scored by Chl followed by n-Hex (1.26 mg/ml), while lowest LC_{90} (2.12 mg/ml) was exhibited by *n*-Hex followed by Chl (7.94 mg/ml). While, the But fraction demonstrated highest LC_{50} (33.5 mg/ml) and LC_{90} (83.61 mg/ml), other fractions showed moderate to higher LC_{50} and LC_{90} values for larval mortality of *Cx. quinquefasciatus*. Our results are in accordance with that of Saidana *et al*., (2007), who established that the chloroform extract of *T. boveana* has strong anti-larval activity against *T. confusum*.

Conclusion

It is inferred from the present study that among the three selected species of genus Tamarix, the methanolic extract of *T. baluchistanica* is more potent for both the antioxidant and larvicidal activities. However, the ethyl acetate fraction of *T. baluchistanica* methanolic extract has highly effective for neutralizing the oxidants i.e. DPPH free radicals and ABTS cations and therefore, needs further screening through bioassays in animal models. While, the *n*-hexane fraction may be exploited in the formulation of biopesticides. The current research also demonstrates for in-depth bioassay guided isolation of the active principles for the vector.

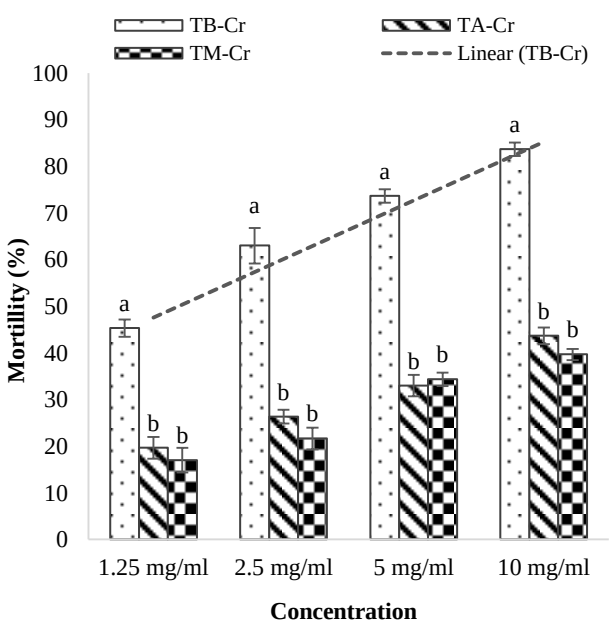

Fig. 5. Larvicidal activity of methanolic crude extracts of aerial parts of *T. baluchistanica, T. androssowii* and *T. mascatensis* against 3rd instar larvae of *Cx. Quinquefasciatus* after 24 h exposure at $p<0.05$. Whereas; TB: *T. baluchistanica*, TA: *T. androssowii*, TM: *T. mascatensis*, Cr: Crude extract.

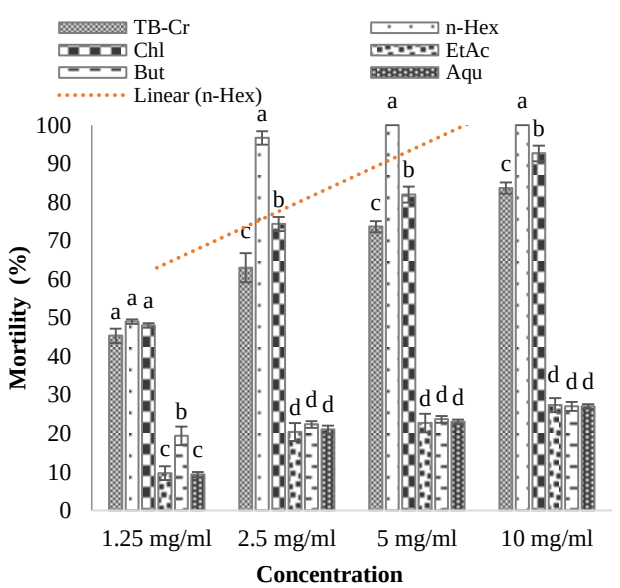

Fig. 6. Larvicidal activity of methanolic crude extracts of aerial parts of *T. baluchistanica* and its fractions in different solvents. All values are mean (± standard error) of three replicates where similar letters are not significantly different at $p<0.05$.TB-Cr: *T. baluchistanica* methanolic extract; *n*-Hex: *n*-Hexane fraction; Chl: chloroform fraction; EtAc: ethyl acetate fraction; But: butanol fraction; Aqu: aqueous fraction.

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