EVALUATION OF ANTIOXIDANT AND LARVICIDAL ACTIVITIES OF SELECTED TAMARIX SPECIES AGAINST THE SOUTHERN HOUSE MOSQUITO “CULEX QUINQUEFASCiATiUS (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-picrylhydrazyl (DPPH), 2', 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonate (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. The chloroform fraction (7.94 mg/ml) of T. baluchistanica showed lowest LC50 of 32.46 µg/ml and IC50 of 91.32 µg/ml against DPPH radicals. This was similar to the standard antioxidant, ascorbic acid which showed IC50 of 32.48 µg/ml and LC50 of 86.73 µg/ml. Antioxidant activity against ABTS demonstrated that among all the fractions, least IC50 of 37.94 µg/ml and LC50 of 129.85 µg/ml was also recorded for ethyl acetate fraction of T. baluchistanica extract, comparable to reference antioxidant, BHT having IC50 of 36.78 µg/ml and IC50 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 LC50 of 0.06 mg/ml, followed by n-hexane fraction having LC50 of 1.26 mg/ml, while lowest LC50 (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 areas of developing countries including Pakistan, there are a lot of medicinal plants based traditional remedies that are used for treatment of various ailments 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 exhibit 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 stem human ailments e.g. malaria, yellow fever, dengue fever, chikungunya, Japanese encephalitis, and lymphatic filariasis etc. (Chowell et al., 2003). 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 Ceratitis capitata. Researchers suggested the antioxidant and larvicidal assessment of Tamarix spp. (Saidana et al., 2007; Rahman 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 (Saidana 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 revealed 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-ethylenobenzothiazoline-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-Baltisttan (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 and coarsely ground with a mechanical grinder. The powdered plant material (10 Kg) was extracted with 80% methanol and dissolved in 100 ml of water. The resulta 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;

\[
\text{Mortality} (\%) = \frac{\text{Number of larve killed}}{\text{Total number of larve}} \times 100
\]

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:

\[
\text{DPPH scavenging(\%)} = \frac{A_0 - A_1}{A_0} \times 100
\]

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 (K₂S₂O₈) 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 IC₉₀ and IC₅₀ (µg/ml) by analyzing data through SPSS 16.0 statistics software. The percent inhibition of ABTS radical was measured, using the following formula:

\[ \text{ABTS scavenging} = \frac{A_0 - A_1}{A_0} \times 100 \]

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 IC₅₀ and IC₉₀ while, in case of larvicidal activity the values were expressed as LC₅₀ and LC₉₀.

**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 IC₉₀ and IC₅₀ 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 IC₅₀ of 32.46 µg/ml and IC₉₀ of 91.32 µg/ml were recorded for EtAc, while similar IC₅₀ of 32.48 µg/ml and IC₉₀ of 86.73 µg/ml was found for As-A. All the other treatment groups showed higher IC₅₀ and IC₉₀ 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 IC₉₀ of promising ethyl acetate fraction (>)90% when compared with standard ascorbic acid (IC₅₀ 4.8 ± 0.54 µg/ml), ethyl acetate fraction showed the best effect (7.25 ± 0.86 µg/ml), with lower IC₅₀ followed by butanol fraction (8.25 ± 0.65 µg/ml) and total extract (45 ± 0.73 µg/ml).

<table>
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<tr>
<th>Groups</th>
<th>DPPH</th>
<th>ABTS</th>
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<tr>
<td></td>
<td>IC₅₀ µg/ml</td>
<td>IC₉₀ µg/ml</td>
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<tr>
<td>Methanolic Extract</td>
<td>77.63</td>
<td>193.19</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>160.82</td>
<td>331.85</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>131.89</td>
<td>293.14</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>32.46</td>
<td>91.32</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>49.1</td>
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<td>Aqueous fraction</td>
<td>185.57</td>
<td>356.59</td>
</tr>
<tr>
<td>Standard</td>
<td>32.48</td>
<td>86.73</td>
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**Table 1. IC₉₀ and IC₅₀(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.**

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 IC50 (99.22 μg/ml) and IC90 (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 IC₅₀ and IC₉₀ values for ABTS cation inhibition are given in Table 1. Among all the plant extracts/fractions minimum IC₉₀ of 37.94 µg/ml and IC₅₀ of 129.85 µg/ml were noted for EtAc, while parallel IC₉₀ of 36.78 µg/ml and IC₅₀ of 113.07 µg/ml was also found for BHT. All the other treatment groups showed higher IC₉₀ and IC₅₀ values for the ABTS cation inhibition. These results are in accordance to the previous reports of Rahuman et al., (2008), Koch 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. LC₅₀ and LC₉₀ of different solvent fractions of methanolic plant extract of T. baluchistanica against, 3rd instar larvae of Cx. quinquefasciatus, after 24 h of exposure.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Larvicidal activity</th>
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<tbody>
<tr>
<td></td>
<td>LC₅₀ mg/ml</td>
</tr>
<tr>
<td>Methanolic Extract</td>
<td>0.76</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>1.26</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>0.06</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>19.46</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>33.55</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>19.8</td>
</tr>
</tbody>
</table>

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 larval killing of C. capitata. Further investigation of fractions of TB-Cr revealed 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 LC₅₀ and LC₉₀ for all the fractions of TB-Cr are given in (Table 2). Lowest LC₅₀ (0.06 mg/ml) was scored by Chl followed by n-Hex (1.26 mg/ml), while lowest LC₉₀ (2.12 mg/ml) was exhibited by n-Hex followed by Chl (7.94 mg/ml). While, the But fraction demonstrated highest LC₅₀ (33.5 mg/ml) and LC₉₀ (83.61 mg/ml), other fractions showed moderate to higher LC₉₀ and LC₅₀ 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. 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.
References


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