Research Article

# THE ASSESSMENT OF *TAMARIX APHYLLA* AND *CALOTROPIS PROCERA* COMPARATIVE AND COMBINE ANTIOXIDANT POTENTIAL

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DOI: 10.38106/LMRJ.2023.5.2-02

Received: 03.06.2023
Accepted: 25.06.2023
Published: 30.06.2023

**ABSTRACT**

Free radicals alter DNA, resulting in various chronic disorders, including cancer. Herbal therapy has a significant potential to block cancer progression and other chronic diseases. The *Tamarix aphylla* plays an essential role in the modulation of free radicals. *Calotropis procera* (leaves), a tropical medicinal plant, has shown protective effects against cancer progression. We have examined the antioxidant therapy of separate or combined forms of the *Tamarix aphylla* and *Calotropis procera* plant extracts. *Tamarix aphylla* demonstrated scavenging activity at various concentrations, including 100 µg/ml, 500 µg/ml, and 1000 µg/ml (29%, 37% and 62%). The scavenging activity of *Calotropis procera* at various concentrations (100 µg/ml, 500 µg/ml, and 1000 µg/ml) was 20%, 31% and 40% against the standard of ascorbic acid (65%, 77% and 84%). The mixture of both plant extracts displayed significant antioxidant potential at various concentrations 100 µg/ml, 500 µg/ml, and 1000 µg/ml which were 45%, 64% and 78%. Our study showed that the mixture of both plants has a significant antioxidant potential by comparing individual plant extracts. Further studies are recommended to elucidate the anti-cancer potential of both plant extracts mixture using in vivo approach for tumor models.

**Key Words**: *Tamarix aphylla*, *Calotropis procera*, Antioxidant activity

## INTRODUCTION

Globally cancer incidence is rising posing a huge burden on healthcare systems. In 2019 the number of cancer patients reported from the United States of America was 1,762,450 new cases and 606,880 cancer deaths (1). Cancer is concerned with daily lifestyle using tobacco, exposure to chemicals, radiation and infectious organisms, as well as some internal factors such as inherited mutations, hormones, immune system disorders and specific random mutations (2). Oxidative stress is the inequity between the production and accumulation of reactive oxygen species (ROS). The reactive oxygen species ROS is one of the main source of many diseases such as cancer, diabetes mellitus, atherosclerosis, and more frequently gynecological diseases especially endometriosis (3-5).

The plants are used for health benefits as a part of traditional folk medicine worldwide. To control the high percentage of various types of cancer needs new anti-cancer drugs (6). Despite the minimal risk of side effects, the possibility of medication interaction cannot be ruled out (7).

*Tamarix aphylla* belongs to the family Tamaricaceae, the most prominent Tamarix species with a height up to 18 m (60 feet). Various local names like saltcedar, Athel tree, Athel tamarisk, and Athel pine know this plant. The plant is currently found in African and Asian countries, and local people use this plant for medicinal purposes, commonly known as tamarisk. It has been reported that *Tamarix aphylla* has strong anti-diabetic potential (8). Recent studies have shown that the *Tamarix aphylla* contains some selective secondary metabolites confirmed by the *Tamarix aphylla* phytochemical screening. The aqueous

and hydroalcoholic extract of *Tamarix aphylla* of different parts confirmed that the *Tamarix aphylla* contains flavonoid glycosides, carboxylic acid steroids, cardiac glycosides, and terpenoids (9, 10). This plant has great potential to work as an anti-diabetic, antibacterial, anti-inflammatory, antifungal and in addition, it also works in periodontal disease, anti-cholinesterase and wound-healing activities. These activities are due to numerous phenolic compounds with astringent effects. In addition to traditional use, the plant has significant anti-diabetic, hypolipidemic, antifungal, antibacterial, cytotoxic, and antioxidant properties (11, 12).

*Calotropis procera* is an evergreen xerophytic plant belonging to the Apocynaceae and subfamily Asclepiadaceae. It is generally found in dry and semi-dry areas. *Calotropis procera* is a multipurpose plant widely used as a traditional medicine in North Africa, the Middle East, South Asia, and South-East Asia (13). *Calotropis procera* is generally used in society meds for treating colds, fever, uncleanliness, asthma, ailment, dermatitis, heartburn, diarrhoea, elephantiasis and skin problems (14).

Several pharmacological activities were performed for various extracts of *Calotropis procera*, like, anticancer, anti-diabetic, cardiovascular, anti-pyretic, anti-cancer, anti-helmintic, hypolipidemic, pain relieving, and anti-convulsant (15, 16). This study investigated the cytotoxic, antioxidant and phytotoxic effect of plants *Tamarix aphylla* extracts in combination with *Calotropis procera* and its resultant fractions

## METHODS

### CHEMICALS FOR BIOLOGICAL ACTIVITIES

2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, and aluminium foil were provided by the Department of Zoology, University of Science and Technology Bannu, Pakistan. Plants fractions and methanolic extract plants were prepared at the Laboratory of the Department of Zoology, University of Science and Technology Bannu Khyber Pakhtunkhwa, Pakistan.

### PLANT MATERIAL AND PREPARATION OF CRUDE EXTRACT

The plant extracts were collected in March 2022 from District Bannu, Khyber Pakhtunkhwa, Pakistan. Fresh, shed-dried leave of *Tamarix aphylla* and *Calotropis procera* plants were grinded into a fine powder with the help of a pestle and mortar. This powder was put into 70% methanol in such a way that the powder was completely submerged in methanol and placed at room temperature for 72 h with frequent agitation. The resulting liquid was filtered by using Whatman No. 3 filter paper. The filtrate was placed at room temperature to evaporate the liquid content. The resulting gummy methanolic extract was put into a falcon tube and lyophilized. The lyophilized sample was stored for further use.

### BIOLOGICAL ASSAYS

#### Antioxidant assay

The published procedure with some modifications was followed for the assay of DPPH free radicals scavenging (17). 100 µl from each of the sample solutions of 100 µg/ml, 500 µg/ml, and 1000 µg/ml were taken. Then the 100 µl was mixed with 900 µl of DPPH solution. The exact process was repeated with 500 µl and the 1000 µl with DPPH solution. The exact process was repeated with the ascorbic acid solution. All these test tubes were incubated at 25°C for about 30 minutes in the dark because of their sensitivity toward light and checked its absorbance on a spectrophotometer at 517nm.

By using the following equation, the potential of the samples to scavenge the DPPH free radicals was calculated;

% DPPH free radicals scavenging effect = (A1-A2/A1)×100

Where A1= the absorbance of DPPH (control) and A2= the absorbance in the presence of samples.

The results were analyzed and presented in graphs.

## RESULTS

The study was designed to explore the antioxidant potential of medicinally important plant extracts such as the leaves of *Tamarix aphylla* and *Calotropis procera.*

### Antioxidant activity of *Tamarix aphylla*

The scavenging activity of the methanolic extract of *Tamarix aphylla* was analyzed in comparison with the standard ascorbic acid using DPPH free radicals assay. By increasing the extract concentration, the scavenging ability also increases, 29% at minimum concentration of 100 µg/mL while 37% and 62% with 500 µg/mL and 1000 µg/mL of *Tamarix aphylla* respectively (Figure 1).

### Antioxidant activity of *Calotropis procera*

The scavenging ability of *Calotropis procera* against free radicals DPPH shows the following different results using different concentrations at 100 µg/ml, 500 µg/ml and 1000 µg/ml. By increasing the extract concentration, the scavenging ability was also increased, which was 20% at minimum concentration while reaches up to 31% and 40% with increasing concentrations of *Calotropis procera* by comparing with control ascorbic acid scavenging activity 65,77 and 84%) as shown in Figure 2.

### Antioxidant activity of mixture of *Tamarix aphylla* and *Calotropis procera*

The combined mixture of both *Tamarix aphylla* and *Calotropis procera* in comparison with the standard ascorbic Acid was performed. The scavenging ability of DPPH and the combined extracts of *Calotropis procera* and *Tamarix aphylla* against free radicals show the following different results with different concentrations which are 100 µg/ml, 500 µg/ml and 1000 µg/ml, respectively. By increasing the extract concentration, the scavenging ability also increases i.e. 45% at minimum concentration while 64% and 78% with a maximum combined concentration of *Tamarix aphylla* and *Calotropis procera* as shown in Figure 3.

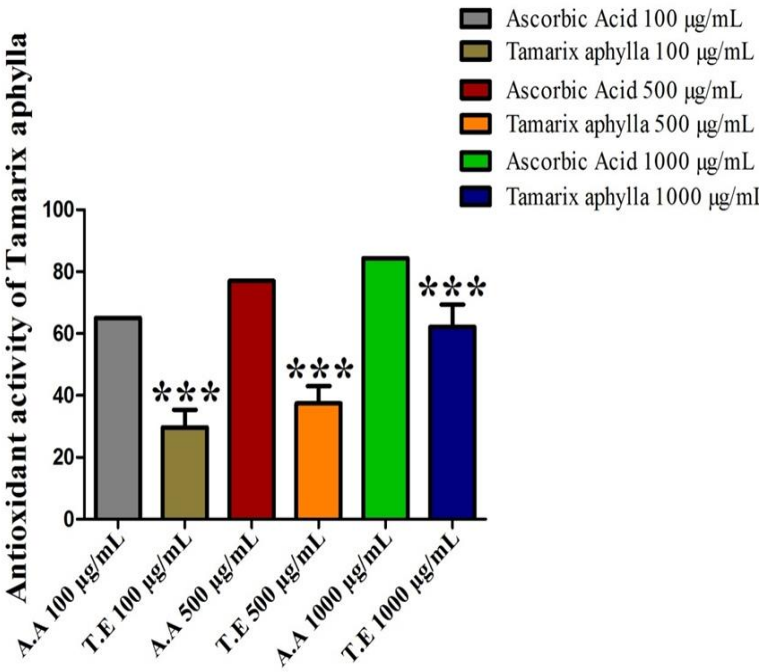


**Figure 1. Antioxidant activity of *Tamarix aphylla***

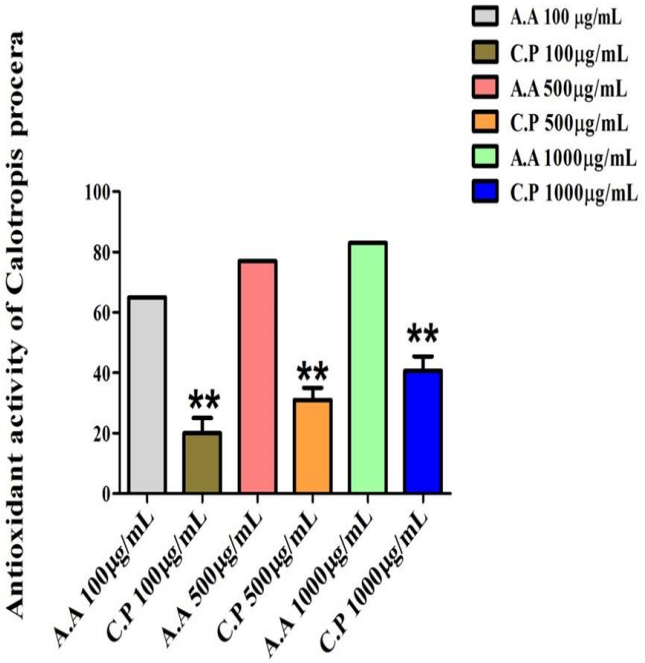


**Figure 2. Antioxidant activity of *Calotropis procera***

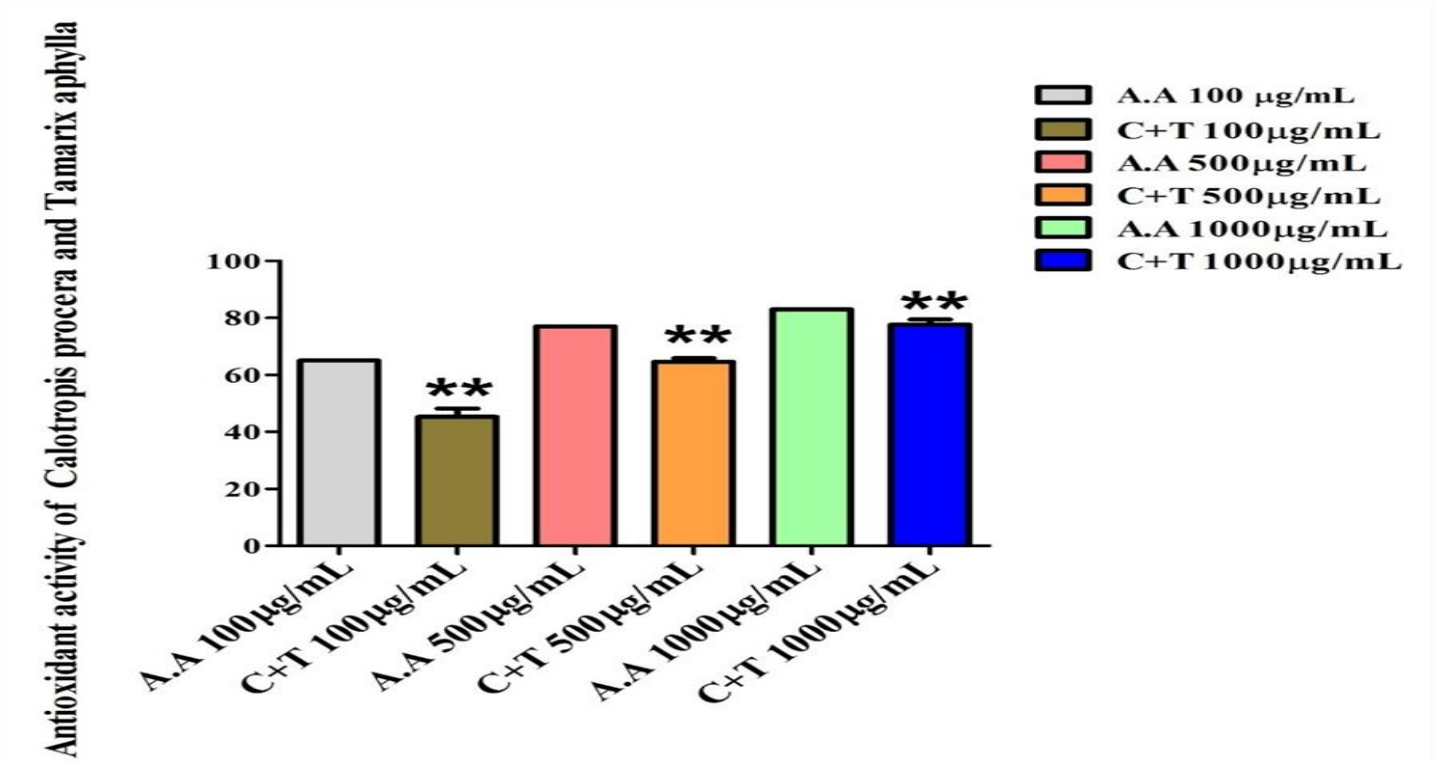


**Figure 3. Antioxidant activity of *Tamarix aphylla* and *Calotropis procera*.**

## DISCUSSION

The mutations that might result from DNA damage induced by free radicals are particularly concerning because they can impair normal cell function and possibly start cancer development. A tumor may develop when the damaged cells continue to divide and expand uncontrollably because the DNA repair mechanisms cannot correct these mutations (18, 19). The overproduced free radicals can directly react with DNA, proteins, and lipids to induce cell abnormalities (20). Free radicals, including superoxide anion, hydroxyl radicals, and hydrogen peroxide, are harmful to cellular components including DNA, lipids and proteins, and are believed to be significant contributors to many diseases such as cancer and cardiovascular diseases (21).

Natural compounds in plants can scavenge free radicals and may have anti-cancer effects. These substances also referred to as phytochemicals or phytonutrients, contain different antioxidants and other bioactive ingredients which has even been proven to reduce the risk of inflammation and illnesses such as diabetes, cardiovascular, neuro-degenerative, microbial-related diseases, and certain types of cancers (22). The findings of new anti-cancer drugs are one of the most emerging research areas of natural products. Further studies are required for the chemical characterization of the active compounds and more comprehensive biological evaluations (23). In several countries, *Tamarix aphylla* is used in traditional medicine for wounds and abscess healing, as an astringent, and for rheumatism and joint pain (24). Our previous published data elucidate that *Tamarix aphylla* has significant antioxidant potential (17).

The *Tamarix aphylla* has a similar antioxidant potential to our published data. The leaves of *Calotropis procera* are used as an antidote for snake bites, rheumatic disorders, viral infection, injuries caused by burns, diarrhoea, and body pain, to cure jaundice and catarrh, antimalarial, anthelmintic and antioxidant activity (25). Next, we examined the antioxidant potential of *Calotropis procera* extracts. The obtained data 20, 31 and 40% showed less significant effects. Furthermore, the mixture of both extracts

displayed considerable antioxidant potential. The mixture of both showed 45, 64 and 78% scavenging activity.
We found that two combined plants had more antioxidant capability than each alone. Further research study needs to elucidate the mechanism.

## CONCLUSION

The present study suggested that the mixture of *Tamarix aphylla* and *Calotropis procera* plants extract showed significant antioxidant potential than individual extracts. Further studies using cell lines and animal models are recommended to highlight potential use of these plants for new drug development in cancer therapeutics.

**Acknowledgement:** This study was carried out by in the research laboratory of Zoology Department, University of Science and Technology Bannu (28100), Khyber Pakhtunkhwa, Pakistan.
**Ethical consideration:** This study was approved by local Research Ethics committee.
**Funding source:** This study required no additional funding
**Conflict of interest:** Authors declare no conflict of Interest

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