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## THE NON-ENZYMATIC ANTIOXIDANT ACTIVITIES OF *FICUS CARICA* L. SUBSP. *CARICA* LEAVES AND ITS ANTIMICROBIAL ACTIVITIES AGAINST FOOD PATHOGENS

G. Okmen\*, O. Turkcan, P. Erdal and D. Isik

Mugla Sitki Kocman University, Faculty of Science, Department of Biology, Mugla, Turkey.

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*Ficus*, Antimicrobial Activity, Antioxidant Activity

### Correspondence to Author:

#### Gulten Okmen

Assistant Professor

Dr., Mugla Sitki Kocman University,  
Faculty of Science, Department of  
Biology, Mugla 48000, Turkey.

E-mail: gultenokmen@gmail.com

**ABSTRACT:** The aim of this work was to investigate of the antimicrobial and antioxidant potentials of methanol extracts from *Ficus carica*. Methanol extracts were screened for antimicrobial activity against different species of Gram positive and Gram negative bacteria and one yeast. The methanol extract of *Ficus carica* showed maximum inhibition zone of 10 mm against *Listeria monocytogenes*. In addition to, the plant extracts were tested against the stable DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical. Trolox was chosen as a standard antioxidant. Finally, the methanol extract displayed a strong antioxidant activity (Trolox equivalent= 1.33 mM). *Ficus carica* methanol extracts have antimicrobial and antioxidant potential.


**INTRODUCTION:** Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments<sup>1</sup>. Medicinal plants have become the focus of intensive study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or are merely based on folklore<sup>2</sup>.

*Ficus carica* Linn. (Syn: *Ficus sycomorou*s; family: *Moraceae*) is commonly reported as "Fig". The cultivated Fig, native to the arid region of Asia Minor, forms a shrub or low-spreading deciduous tree.

The large, wavy-margined leaves are usually 5 lobed but may have only 4 or 3 lobes<sup>3</sup>. *F. carica* has been reported to have numerous bioactive compounds such as arabinose,  $\beta$ -amyrins,  $\beta$ -carotenes, glycosides, and xanthotoxol<sup>4-6</sup>.

Many compounds have been isolated from aerial roots, bark, leaves and fruits of *Ficus* sp. and some compounds showed piscicidal, germination inhibitory and antifungal activities, etc.<sup>7-11</sup>. It is well known that many natural substances in plants have antioxidant activity. Of which, phenolics were one of the most notable groups. In recent years, much research has focused on antioxidant activity of phenolic compounds in traditional medicinal plants, and a positive correlation was observed between the high phenolic content and the strong antioxidant activity<sup>12, 13</sup>. In addition to, medicinal plants represent a rich source of are antimicrobial agents. These plants are used medicinally in different countries and are a source of many potent and powerful drugs<sup>14</sup>. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy<sup>15</sup>.

Many plants have been used due to their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The antimicrobial activity of *Ficus carica* has not been studied against food pathogens, the *in vitro* antimicrobial activity of leaves parts of the

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plant growing in Mugla was evaluated using disc diffusion method. However, a few reports have been published on the antioxidant activity of *Ficus carica*. In this study, methanol extracts of the plant leaves were investigated for antimicrobial, and antioxidant activities.

#### MATERIAL AND METHODS:

**Plant material:** *Ficus carica* samples were collected from Mugla Sitki Kocman University Campus at July in 2012, Mugla, Turkey. Taxonomical identification of plant was performed by Olcay Ceylan from the Mugla Sitki Kocman University, Turkey and a specimen was deposited in the herbarium of the Biology Department of Mugla Sitki Kocman University. The identification of these specimens was carried out using the Flora of Turkey<sup>16</sup>. The leaves were washed thoroughly 2-3 times with running water and once with sterilized distilled water. Fresh plant material was air-dried. The dried leaves were powdered in a laboratory mill. All samples were stored at ambient temperature until initial sample preparation, after which they were stored at 4°C until required for analysis.

**Plant extraction:** The air dried and powdered leaves were extracted with methanol (100mg/mL) using the Soxhlet apparatus. The extract was evaporated and then extracted in methanol and then kept in small sterilized opac bottles under refrigerated conditions until used.

**Microorganisms and cultivation:** The plant extracts were individually tested against food pathogens. These include; *Bacillus subtilis* RSKK 245, *Staphylococcus aureus* RSKK 2392, *Salmonella Typhimurium* RSKK 19, *Enterococcus faecalis* ATCC 8093, *Escherichia coli* ATCC 11229, *Listeria monocytogenes* ATCC 7644, *Yersinia enterocolitica* NCTC 11174 and *Candida albicans* RSKK 02029. The bacteria were grown for 24h at 37°C in Mueller- Hinton Broth (Merck). *C. albicans* was grown for 24h at 30°C in Sabouraud Dextrose Broth (Merck). These strains of bacteria and *C. albicans* were obtained from ATCC (American Type Culture Collection, USA), RSKK (Refik Saydam National Type Culture Collection, Turkey) or NCTC (National Collection of Type Cultures).

**Antimicrobial activity assay:** Bauer-Kirby method applied for antimicrobial activity<sup>17</sup>. The leaf methanol extract was tested by disc diffusion assay. The bacteria were maintained on Mueller-Hinton agar plates (MHA, Merck) at 37°C. In addition to, yeast was maintained on Sabouraud Dextrose agar plates (SDA, Merck). Bacterial and yeast cultures adjusted to 0, 5 McFarland. Incubations were at 37°C for 24- 48 h for bacteria. Temperature adjusted for *C. albicans* was at 30°C for 24- 48 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zones around the discs after 24 h. Methanol used as negative control. Nystatin (100µg) and ampicillin (10µg) antibiotics used as positive controls. All tests were performed in triplicate and the mean values were given.

**Determination of minimum inhibitory concentration (MIC):** The MIC was evaluated on plant extracts as antimicrobial activity. The MIC was taken as the lowest concentration that inhibited growth after incubation. The broth dilution assay was performed as described in the CLSI standards<sup>18, 19</sup>. This test were performed at final concentrations of the extract (6500; 3250; 1625; 812; 406 µg/mL).

**Determination of non-enzymatic antioxidant activity:** The non-enzymatic antioxidant activity was determined using DPPH as a free radical. The stable DPPH was used for determination of free radical-scavenging activity of the extracts. Extract (0.1 mL) was added to 3.9 mL of a 0.1 mM methanol DPPH solution. After incubation for 30 minutes, absorbance of extract was measured at 515 nm using spectrophotometer. Methanol was used as a blank, while methanol with DPPH solution was used as a control<sup>20</sup>. Trolox was used for reference antioxidant. The DPPH scavenging capacity expressed in percentage (%) was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{extract})}}{\text{Abs}_{(\text{control})}} \right] \times 100.$$

Where Abs<sub>(control)</sub> is the absorbance value of the DPPH- blank sample and Abs<sub>(extract)</sub> is the absorbance value of the test solution.

**RESULTS:**

**Antimicrobial activity:** The antimicrobial activities of the plant methanol extracts were evaluated *in-vitro* against different microorganisms, which are known to cause some diseases in foods. Results of antimicrobial activities of methanol extracts of used plant against the test microorganisms are shown in **Table 1**.

The results of antimicrobial activities were recorded as zone of inhibition in mm for all the materials used as follows. Results show that the plant methanol extracts inhibited the growths of five bacteria and the inhibition zones ranged between 7-10 mm. In addition the methanolic extract of this plant did not determine any anticandidal effects against used yeast. Furthermore, methanol extract of *Ficus carica* did not show any antibacterial effects against used two bacteria. Results also show that the methanol extracts of *Ficus carica* inhibited the growth of 4 bacteria with 7 mm inhibition zones (**Table 1**).

**TABLE 1: ANTIMICROBIAL ACTIVITIES OF METHANOL EXTRACTS OF *FICUS CARICA***

Microorganisms	Inhibition zone diameters (mm)		
	Plant extract (100 mg/ml)	Antibiotics N	Antibiotics A
<i>B. subtilis</i> RSKK 245	-	(nt)	10
<i>S. aureus</i> RSKK 2392	7	(nt)	10
<i>S. Typhimurium</i> RSKK 19	7	(nt)	(nt)
<i>E. faecalis</i> ATCC 8093	7	(nt)	(-)
<i>E. coli</i> ATCC 11229	-	(nt)	(nt)
<i>L. monocytogenes</i> ATCC 7644	10	(nt)	12
<i>Y. enterocolitica</i> NCTC 11174	7	(nt)	(nt)
<i>C. albicans</i> RSKK 02029	-	7	(nt)

N: nystatin (100 µg); A: ampicillin (10µg);  
(-): zone did not occur (nt): not tested

Methanol extracts of the leaves were found to be effective against all of the Gram positive and negative bacteria, except three microorganisms (**Table 1**). The maximum zone of inhibition was produced by methanol extract against *Listeria monocytogenes* ATCC 7644 (10mm). Whereas, the inhibition zone was not produced by the extract against *Bacillus subtilis* RSKK 245, *Escherichia coli* ATCC 11229 and *Candida albicans* RSKK 02029. These microorganisms were found resistant

to the methanol extract. The extract was found equally effective in inhibiting the growths of *Staphylococcus aureus* RSKK 2392, *Salmonella Typhimurium* RSKK 19, *Enterococcus faecalis* ATCC 8093, and *Yersinia enterocolitica* NCTC 11174. Nystatin (100µg) and ampicillin (10µg), antibiotics used as positive control. Ampicillin strongly was inhibited the growth of *Listeria monocytogenes* ATCC 7644 (**Table 1**).

**MIC tests:** **Table 2** shows MICs of *Ficus carica* leaf extracts obtained by the broth dilution method. All of bacterial strains showed the lowest sensitivity at 3250 µg /mL methanol extract, except *Yersinia enterocolitica* NCTC 11174 (**Table 2**).

**TABLE 2: MINIMUM INHIBITORY CONCENTRATIONS OF METHANOLIC EXTRACTS OF *FICUS CARICA***

Microorganisms	MIC (µg/mL)
<i>Bacillus subtilis</i> RSKK 245	3250
<i>Staphylococcus aureus</i> RSKK 2392	3250
<i>Salmonella typhimurium</i> RSKK 19	3250
<i>Enterococcus faecalis</i> ATCC 8093	3250
<i>Escherichia coli</i> ATCC 11229	3250
<i>Listeria monocytogenes</i> ATCC 7644	3250
<i>Yersinia enterocolitica</i> NCTC 11174	6500
<i>Candida albicans</i> RSKK 02029	3250

**Non-enzymatic antioxidant activity:** The non-enzymatic antioxidant activity of the plant extract was evaluated by the DPPH radical scavenging capacity. Table 3 shows the percent of DPPH radical scavenging capacity with trolox as reference. The extract showed 52 % inhibition at 100 mg/mL extract concentration. Trolox equivalent value was 1.33 mM/g DW (**Table 3**).

**TABLE 3: DPPH RADICAL SCAVENGING CAPACITY OF *FICUS CARICA* LEAF EXTRACT**

DPPH radical scavenge (%)	Trolox equivalent (mM (TE) /g DW)
52 ± 0.09	1.33

TE: Trolox equivalent; DW: dry weight

**DISCUSSION:** This study confirms that the leaf of *Ficus carica* posses antimicrobial and antioxidant activities. The properties commonly found in plants, and they have been reported to have multiple biological effects including antimicrobial, antiviral and antioxidant activities. Medicinal

plants have been traditionally used worldwide for the treatment of various diseases<sup>21</sup>. They have proved to be abundant sources of biologically active compounds, many of which have been used as compounds to develop new pharmaceuticals<sup>22</sup>. This genus was selected based on their relevant ethnomedical use<sup>23-25</sup>.

Food pathogens have caused considerable concern for the food industry, health regulatory officials, and consumers<sup>26</sup>. *L. monocytogenes* is a food borne pathogen responsible for the disease listeriosis. Listeriosis is primarily transmitted through various foods, fish, dairy products, cured or processed meat, egg, poultry, seafood, salad, fruits and vegetables<sup>27</sup>. A severe infection, listeriosis has been associated with a mortality rate as high as 30-40%<sup>28</sup>. In the present study, extracts of the plant leaves obtained in methanol solvents were tested against the test organisms. The antimicrobial activity was compared with the standard antibiotics.

As a result, the methanol extract showed maximum inhibition against *Listeria monocytogenes* ATCC 7644 (**Table 1**). Shan et al.<sup>29</sup> reported a highly positive relationship ( $R^2=0.73$ ) between antibacterial activity and phenolic content of the tested extracts against *L. monocytogenes*. According to a report of Rasooli et al.<sup>30</sup> reported that various concentrations of essential oils from *Thymus eriocalyx* and *Thymus x-porlock* tested on agar plates and in broth tubes showed very strong anti-*listeria* properties. Pirbalouti et al.<sup>31</sup> found that plant extracts and essential oils produced inhibition zone of 8–18 mm against the *L. monocytogenes*. Similar results with different extracts were obtained by Karmegam et al.<sup>32</sup>.

In this study, the inhibition zone was not produced by methanol extract against *Bacillus subtilis* RSKK 245, *Escherichia coli* ATCC 11229 and *Candida albicans* RSKK 02029 (Table 1). Kubmarawa et al.<sup>33</sup> reported that the extract of *Ficus platyphylla* was not inhibited *B. subtilis* and *E. coli*. Both of bacteria were developed resistance against the plant extract. Uguz<sup>34</sup> determined that the extract of *Ficus carica* subsp. *carica* was not inhibited three test fungi. These reports also support the results we obtained from our study.

The extract was found lowly effective in inhibiting the growth of *Staphylococcus aureus* RSKK 2392, *Salmonella* Typhimurium RSKK 19, *Enterococcus faecalis* ATCC 8093, and *Yersinia enterocolitica* NCTC 11174 (**Table 1**). Similar results with four *Ficus* sp. extract were obtained by Nair and Chanda<sup>35</sup>.

According to this study, all of bacterial strains showed the lowest sensitivity at 3250µg/mL methanol extract, except *Yersinia enterocolitica* NCTC 11174 (**Table 2**). AlSabri et al.<sup>36</sup> reported that minimum inhibitory concentration value of *Arbutus pavarii* against *S. aureus* was found as 4.86mg/mL. In this study, MIC value was generally measured as 3250µg/mL, and our results are better than those of AlSabri et al.<sup>36</sup>. The effects of the methanol extracts against test bacteria are of weak activity.

Metabolism in majority of complex living organisms requires oxygen for its survival. But, oxygen being, a highly reactive molecule damages living organisms by producing reactive oxygen species<sup>37</sup>. An antioxidant is a molecule that slows or prevents the oxidation of the molecules<sup>38</sup>. It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, tissue inflammatory, cardiovascular disease<sup>12</sup>. The results of DPPH scavenging assay of *Ficus carica* extracts are shown in **Table 3**. The extract showed 52 % inhibition at 100 mg/mL extract concentration (**Table 3**). Oliveira, et al. (2009) reported that *F. carica* showed the presence of phenolics and organic acids<sup>39</sup>. Phenolic compounds include 3-O- and 5-ocaffeoylquinic acids, ferulic acid, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, psoralen and bergapten. Organic acids in leaves include oxalic, citric, malic, quinic, shikimic and fumaric acids. Leaves possess the strongest antioxidant potential and pulp the weakest one. These facts may be explained by the highest amounts of phenolic compounds occurring in leaves<sup>39</sup>.

Our findings suggest that *Ficus carica* has significant antibacterial activity and it could be very useful in the discovery of novel antibacterial agents of plant origin. The species due to their strong antioxidant and biological properties are also

known to diffuse the free radical and can be used as a possible food additive and pharmaceutical industries. In conclusion, *Ficus carica* might be considered as a potential source of metabolites which could be developed as precursors for antimicrobial and antioxidants drugs. After this study, further work should be performed to describe the antimicrobial activities in more detail as well as their activity *in vivo*. Also phytochemical studies will be necessary to isolate the active constituents and evaluate the antibacterial activities against a wide range of bacterial populations.

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