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# Antibacterial and antioxidant activity of flavonoid, glycoside and alkaloid extracts of *Tilia Cordata*

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**Abstract**---About 12,000 chemical compounds isolated from medicinal plants and used to conduct crucial biological functions. These phytochemicals offer long-term health benefits for humans, thus they can be used effectively for treat a variety of human ailments. The goal of this study was to assess the in vitro free radical scavenging and antibacterial activities of glycoside, flavonoid and alkaloid extracts of *Tilia cordata* plant. According to the findings, flavonoid extract exhibits strong inhibitory action against *Staphylococcus aureus* but no activity against *Escherichia coli*. The free radical scavenging activity exhibited that the flavonoid extract was more effective in inhibiting lipid peroxidation more than alkaloid extract, whereas the glycoside extract had no antioxidant activity.

**Keywords**---*tilia cordata*, zezafoon, antibacterial activity, antioxidant activity.

**Introduction**

*Tilia* species are known for their fragrant flowers and deciduous trees with simple alternating leaves. The dried inflorescences of various species are commonly used to make natural tea. *Tilia* species are important plants in traditional medicine because of their therapeutic properties [1,2]. *Tilia cordata* (also known as lime tree or Zezafoon) belongs to *Malvaceae* family. The plant is inherent to Europe,

favoring warmer regions, and spread from the Caucasus to north-west Spain and Wales as well as from southern Finland to southern Italy [3,4]. It has been a part of European forests for over six millennia [3,4]. The secondary metabolites found in lime tree include various compounds such as quercetin, chlorogenic acids, p-coumaric, kaempferol, caffeic, myricetin, quercitrin, citral, eugenol, anethole, hyperoside,  $\alpha$ - and  $\beta$ -thujone, isoquercitrin, limonene, farnesol, fenchone, terpineol,  $\alpha$ -pinene, nerol, citronellol, and menthone. The flowers of *Tilia cordata* contain flavonoids, volatile oils, and tannins and are widely used for the curing of anxiety and fever. The leaves of *Tilia cordata* are traditionally equipped as a herbal tea to ease the symptoms of common catarrh, coughs, and colds [5,6,7]. According to the British Herbal Pharmacopeia, the flower of *Tilia cordata* is beneficial in the treatment of hypertension induced by nerve stresses and arteriosclerosis. Also, *Tilia cordata* has been shown to have anti-proliferative effect in lymphoma cell lines and the murine lymphoma cell line BW 5147 in previous investigations [2,8].

## **Materials and Methods**

### **Plant and reagents**

The plant leaves were purchased from a market of medical plants. The leaves was taken and pulverized with a manual mill, and the powder was stored until it was needed. Sigma-Aldrich provided all of the reagents and solvents utilized.

### **Ethanolic extract of *Tilia cordata***

Five grams of ground plant leaves were stirred for 12 hours at room temperature with 50 ml of 70% ethanol. The residue filtered out and the filtrate employed in the preliminary qualitative tests [9].

### **Flavonoids extraction**

25gm of plant leaves powder was combined with 70% methanol and stirred for 24 hours at room temperature, after that the extract was filtered, 1% aqueous lead acetate was added and the filtration was used to separate the precipitate, which was then treated with 50 mL acetone and 10 mL 2N HCl. The mixture was then filtered, and the filtrate left at ambient temperature to dry. The weight of the produced amorphous powder was 0.2gm, which was dissolved in 25 ml water and extracted twice using ethyl acetate (25ml), the collected ethyl acetate fractions being dried to yield 0.1gm [10].

### **Alkaloids extraction**

On a water bath, 20 gram of dried plant leaves were heated with 250 ml of (10%Acetic acid in ethanol) for 24 hours. Filtration was used to remove the residue, and the filtrate was concentrated to 15ml before being acidified with 2% sulphuric acid. After basification with ammonium hydroxide to pH 9, the mixture was extracted with chloroform (25ml) three times. The collected chloroform layers were vaporized to obtained 0.1 gm[11].

### Glycosides extraction

20 gm dried plant leaves was mixed with 150 ml 2% acetic acid, boiled over a water bath for 8 hours with stirring, then filtered, and the filtrate was extracted with n-butanol saturated with sodium chloride. The butanol fraction was vaporized in air to get 0.2 gram [12].

### Antimicrobial activity

The *in vitro* antibacterial characteristics of the three extracts were tested by agar well diffusion method. *Escherichia coli*, a Gram-negative bacterium and *Staphylococcus aureus*, a Gram-positive bacteria, were among the selected microbes. Summarize the method as follows: On the surface of Nutrient agar N.A medium, 0.2 mL of bacterial inocula were put and 0.03g /mL of tested extracts and amoxicillin (as reference drug) were dissolved in dimethyl sulfoxide and deposited in central pore in plates and incubated at (37 ± 2°C), the inhibition zones of both bacteria were assessed in millimeter unit [13].

### Antioxidant activity

The anti-oxidant properties of extracts established using the β-carotene bleaching assay. In a combination 0.2 ml Tween 20 of and 0.02 ml linoleic acid, 1 ml - carotene (0.2 mg / ml in chloroform) was added. After vaporized the chloroform, 50 ml distilled water was added, and 3.8 ml of the combination was mixed with 0.2 ml of the examined extracts and the reference substance (butylated hydroxyl toluene BHT) and the absorbance recorded at 470 nm after mixing. The samples were then exposed to thermal autoxidation at 45 °C for 2 hours in a water bath and the absorbance was recorded every 15 minutes [14]. The following equation was used to compute antioxidant activity (AA):

$$\%AA = 1 - [ Ai - At / *Ai - *At ] \times 100$$

Where Ai: the first absorbance of tester, At: the last absorbance of tester, \*Ai : the first absorbance of control, \*At : the last absorbance of control.

### Result and Discussion

Table 1 indicate the results of preliminary phytochemical analysis of alcoholic extract of *Tilia cordata*.

Table 1  
Preliminary qualitative tests for ethanolic extract

Active part	Flavonoid s	Saponin s	Alkaloid s	Tannin s	Protien s	Ninhydri n	Terpenoid s	Glycosid e
Result	+	+	+	-	-	+	-	+

Absence = - ; Presence = +

### Antimicrobial activity

Table 2 shows the outcomes of *in vitro* antibacterial activity of the three extracts against selected bacteria (*E. coli* and *S. aureus*) using the well agar diffusion method. The flavonoid extract had a significant inhibitory zone against *S. aureus* which was 18mm larger than the conventional antibiotic amoxicillin (12mm), but the alkaloid and glycoside extracts had no effect on the microorganisms. Herbal efficacy against bacteria may be owing to the occurrence of active phytochemicals such as alkaloids, phenolics, and terpenoids that have been shown to have antibacterial properties. Membrane-active chemicals are often bactericidal within minutes due to interactions with bacterial membranes, so that numerous constituents of medicinal plants have been utilized to treat infectious diseases [15,16]. Flavonoids, which have a C6-C3-C6 structure, are the most common polyphenols. Until recently, there have been over 8000 flavonoid molecules identified. The majority of them have demonstrated numerous biological functions such as anticancer, antibiosis, cardiovascular protection, anti-oxidation and antiinflammation. It is worth noting that some flavonoids make bacteria more sensitive to antibiotics and due to their antibacterial qualities, flavonoids have been recommended for the creation of new medicines for the cure of various microbial illnesses, in light of the rise in microbial resistance to antibiotics [17-19].

Flavonoids' antibacterial action is determined by their structures, specifically the substitutions on aromatic rings. With the increasing discovery of plant extracts containing antibacterial properties, more and more flavonoids, particularly those with hydrophobic substituents, have been revealed to be antibacterial agents. Flavonoids kill bacteria in three ways: reducing bacterial pathogenicity, activating antibiotics synergistically, and directly killing bacteria [19,20]. In another review, flavonoids' antibacterial activity was thought to be mediated by inhibiting cell membrane formation and having an aggregatory impact on entire bacterial cells [21]. The flavonoid extract has good efficacy against gram-positive bacteria *Staphylococcus aureus* but none against gram-negative bacteria *Escherichia coli*. This could be linked to *E.coli's* outer membrane, which is more resistant to morphological alterations generated by the extract. The specific structural components of gram-negative bacteria obstruct access to bioactive chemicals and prevent them from breaking the cell wall. In other words, gram-positive bacteria's cell walls are less sophisticated and thicker than gram-negative bacteria's, allowing some substances to get through [22].

Table 2  
The Inhibition Zones of F, A and G extracts Against Selected Bacteria

Sample	<i>S. aureus</i>	<i>E.coli</i>
A (alkaloid)	0	0
F (flavonoid)	18	0
G (glycoside)	0	0
Amoxicillin	12	15

### Antioxidant activity

The activity of the isolated extracts as antioxidant was valued using the  $\beta$  – carotene bleaching method based on the correlation between time and absorbance as shown in Table -3- and figure-1- and compared to BHT, which was used as a reference. The result exhibited that the flavonoid extract has (31 %) activity in inhibition lipid peroxidation compared to the typical antioxidant BHT (73 %) and the alkaloid extract has (10.6 %) antioxidant activity, whereas the glycoside extract had no effect. Herbs are plants that are used as both medicine and food because they comprise a significant amount of natural antioxidants. Flavonoids, phenols, and tannins are natural antioxidants that protect against diseases caused by oxidative stress, such as inflammation, cancer, diabetes and others [23]. Plant flavonoids are the more powerful antioxidants with the ability to reduce the risk of aging, cancer, and free superoxide radical damage. A wide spectrum of flavonoids minimize the negative effects of oxidative processes on organic molecules such as lipids, carbohydrates, proteins, and DNA in biological systems. Flavonoids' antioxidant activity is dual in that it scavenges ROS and RNS and also inhibiting oxidases [24-26]. Radicals oxidize flavonoids, resulting in a less reactive, more stable radical. Radicals are rendered inactive due to the strong reactivity of the flavonoids' hydroxyl group. According to the below equation, the free hydroxyl group will give its hydrogen atom to a radical molecule, stabilizing it and generating a relatively stable flavonoid phenoxyl radical [27,28].  $R\cdot + \text{flavonoid (OH)} = \text{RH} + \text{flavonoid(O}\cdot\text{)}$

Table 3  
Efficiency results of extracts as antioxidants

sample	Ai	At	*Ai	*At	AA%
BHT	2.436	2.364	2.078	1.813	73
A( alkaloid)	2.191	1.954	2.078	1.813	10.6
F (flavonoid)	2.073	1.89	2.078	1.813	31

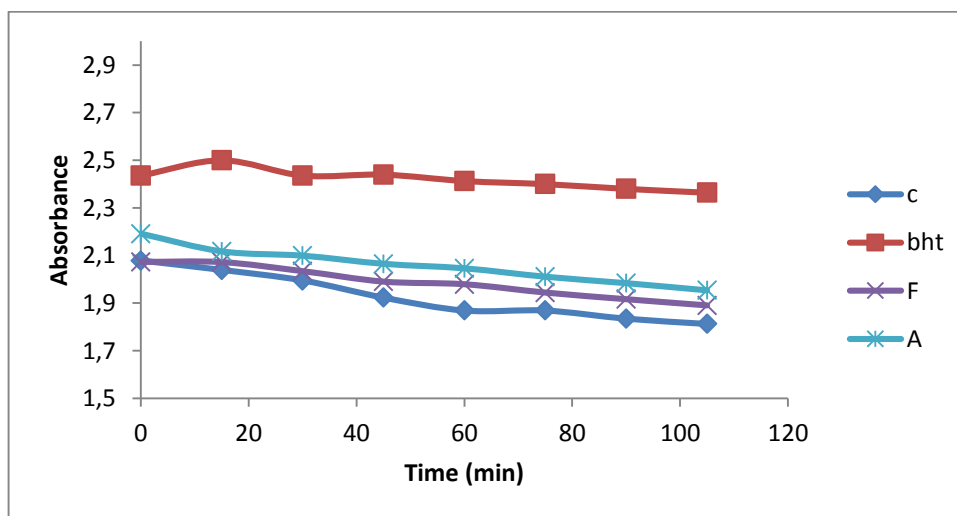


Fig 1. Antioxidant activity of flavonoid (F) and alkaloid (A) extracts

## Conclusion

Briefly, in this study the effectiveness of alkaloid, flavonoid and glycoside extracts of *Tilia cordata* were evaluated as anti-bacterial and antioxidants. The results showed that the flavonoid extract was effective against *S. aureus* bacteria and this extract showed good efficacy as an anti-free radical. Therefore, the study recommends conducting toxicity tests for this extract in vivo for the possibility of using it as natural antibiotic and as a natural antioxidant.

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