Research Article

Assessments of Total Flavonoids, Anti-oxidant and Antibacterial Activity of *Ficus religiosa* Methanolic Extract *in vitro*.

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ABSTRACT

Herbs are plants that are valued for their medicinal and savory qualities. *Ficus religiosa* is very necessary medicinal plant used to treat different diseases including mastitis, otitis media, dysmenorrhea and diabetis. The present study was conducted to evaluate total flavonoids, anti-oxidant and antibacterial activity of *Ficus religiosa* methanolic extract *in vitro.* Total flavonoids content was spectrophotochemically determined in the extract, and it was 113.1±16.4µg/ml. In addition, anti-oxidant activity of *Ficus religiosa* methanolic extract was evaluated *in vitro* via assessment of reductive ability and DPPH radical scavenging activity. At all concentrations tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) in reductive ability, the absorbance was significantly increased in a concentration-dependent manner. Moreover, the extract was significantly more effective in DPPH radical scavenging activity than vitamin C at the four concentrations tested (0.0625, 0.125, 0.250 and 0.500 mg/ml).The results of antibacterial showed that methanolic extract at lower concentrations(200) mg/ml have moderate antibacterial activity against some pathogenic bacteria in which the diameter of zone of inhibition range from (15-16)mm for *Streptpyogens* and (14-16)mm for the *Staph aureus,* and to range from (17-22)mm for *Streptpyogens* and from (16-19) for *Staph aureus* at concentration (200,300 mg/ml). The results of *Pseudomonas aeruginosa* and *Enterobacters sp* reveals that it was resistant to the concentration (200,300)mg/ml of plant extract while it's have moderate zone of inhibition at high concentration (400)mg/ml in which zone of inhibition range from (13-14)mm for *Pseudomonas aeruginosa* and from (11-13)mm for *Enterobacter spp* as. While *Escherichia coli* were resistant to extract at all concentration. The methanol extract of *f.religiosa* is rich in flavonoids, and such richness may have potentiated the extract to have strong anti-oxidant, radical scavenging activities and antibacterial activity *in vitro*.

Keywords: *Ficus reliogosa*, Total flavonoid, Antioxidant, Antibacterial.

INTRODUCTION

Over the past decades, herbal medicine has become a topic of global importance, making an impact on both world health and international trade[1]. Medicinal plants continue to play a central role in the health care system of large proportions of the world's population (Barens, 2002). Medicinal plants have been used as a source of medicine to treat illness since time immemorial (Shoeb, 2006). Active compound present in the medicinal plants provide the bountiful resource of active compounds for the pharmaceutical, cosmetics and food industries, and more recently in agriculture for pest control (Csekeetal., 2006).Generally herbs are containing alkaloids, flavones, antioxidants, xanthones, omega-3 fatty acids, vitamins, minerals and fibers. Research recorded that herbs are derivative from plants and they act as controlling biochemical metabolites either by direct intermediary metabolism or regulating cancer pathways and encouraging immunity (Sharma *et al*., 2010).

Pharmacological studies carried out on the plant materials of *Ficu sreligiosa* provide a practical support for its various traditional consumptions (Aiyegoro and Okoh, 2009). Singh and his colleges (2011) suggested a detailed analysis for the activities of the plant as anticancer drug, cardiovascular, neuron inflammatory and parasitic infections (Sheetal *et al*., 2008). Pharmacological studies were aimed to certify its traditional uses for wound healing, anti-bacterial and anti-anxiety activity (Taskeen *et al*., 2009)

Ficus (Moraceae) considered as one of the largest genera of angiosperms which had 800 species of trees, epiphytes and shrubs in the tropical and subtropical regions around the world (Loutfy *et al*., 2005). It is one of the most assorted plant genera with respect to its growth environment with both deciduous and perennial free standing trees, stranglers, climbers, small shrubs and lithophytes (Ronstadt *al*., 2008). Ficus spp. commonly known as fig, tree of Peepul, tree of Pipaland sacred fig (Khare, 2007), is a small or moderately size deciduous tree indigenous to Persia, Asia, Minor, Syria, Iraq and Mediterranean region, and widely found in tropical and subtropical regions of India (AL-Yousuf, 2012). The leaf aqueous and alchoholic extracts of *F. religiosa* characterized as antibacterial effect against *Bacillus subtilis, Pseudomonas aeruginosa, E .coli* and *Salmonella typhi* (Preethi *et al*., 2010).

MATERIALS AND METHODS

Collection of *Ficus religiosa*

Ficus religiosa leaves were collected from garden in Baghdad University, Iraq, during the period November - 2016.

They were identified by Doctor Ali Al-Mosawy/ Plant Taxonomy/ University of Baghdad/ Baghdad/ Iraq. The fresh leaves of *F. religiosa* were collected and wash away by tap water to remove dust then let's to dry by air dry. They were grained to be powder.

Preparation of Plant extraction

The extract was prepared according to method presented by (Taskeen *et al*., 2009)**.** Fifty grams powder was soaked in (300 ml) of 80% methanol, for one hour in sonication, then after 24 hours of stirring the mixture was then filtered by a Buchner funnel under vacuum pressure repeated two times. Then, dried using a rotary evaporator under vacuum, and stored under sterile conditions in cool place at -20°C until use.

Determination of Total Flavonoids

Spectrophotochemically method was used to determine total flavonoids in *F. religiosa* methanolic extract by using rutin as standard depending on aluminium chloride colorimetric method as described by Sakanaka *et al*., (2005). The plant extract (3.2 mg) was dissolved in 5 ml of 50% methanol, and then 1 ml of a 5% (w/v) sodium nitrite solution was added. Aluminium chloride solution10% (w/v) was added to all tubes about1 ml to the mixture and leaved for 5 minutes, then 10ml of NaOH10% (w/v) solution was added. Then complete the mixture volume up to 50 ml with distilled water and mixed very well. Finally, after 15 min, the absorbance was measured at 450 nm by using spectrophotometer. A six concentration of rutin were used from which a standard curve was prepared (2.5, 5, 10, 20, 40 and 80 µg). The total flavonoids content was determined using a curve-fitting equation of the standard curve.

Assessment of Anti-oxidant Activity *in vitro*

Determination of Reductive Ability

Fua*et al*. (2010) method was used to evaluate the reductive ability of the plant extract, 1 ml of different concentrations of the plant methanolic extract (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) was mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of potassium ferricyanide1%, and then incubated at 50°C for about 20 minutes. After that, 1ml of trichloroacetic acid 10% was added to stop the reaction. After centrifugation 10 minutes at 3000 rpm, 2 ml of distilled water and 0.5 ml of freshly prepared 1% Ferric chloride were added to 2.5 ml of the supernatant and mixed well. Finally, the absorbance was measured at 700nm. Same procedure was done to the Trolox solutions (standards). All tests were done in triplicates.

Determination of DPPH Radical Scavenging Activity

The method of Sanja *et al*., (2009) was done. 0.1 ml of the methanolic extract of plant or standards (0.625, 0.125, 0.250 and 0.500 mg/ml) was added to 3.9 ml of DPPH solution in a test tube to reach to about 4 ml as a final volume. After incubation for 30 minutes at 37°C, the absorbance was determined at 517 nm using spectrophotometer. Triplicates measurements were made for all concentrations. The scavenge ability of DPPH free radical was determined by the following equation (Abbood *et al*., 2015):

$$\text{DPPH radical scavenging activity}(\%) = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100$$

- **Determination of Antibacterial activity**

For detection antibacterial activity of methanolic extract of *F. religiosa* against different pathogenic bacteria (*Staph aureus, Streptpyogens, Pseudomonas aeruginosa, Enterobacterssp and Escherichia coli*) which were isolated from (urinary tract infections) UTI patients. Different concentration of the plant methanolic extract (200,300,400mg/ml) in addition to negative control were used.

RESULTS

Total Flavonoids Content

Total flavonoids content were spectrophotochemically determined in *Ficus religiosa* leaf methanolic extract of as rutin equivalent. The plant methanolic extract was found to contain 113.1±16.4µg/ml flavonoids.

Anti-oxidant and Radical Scavenging Activity

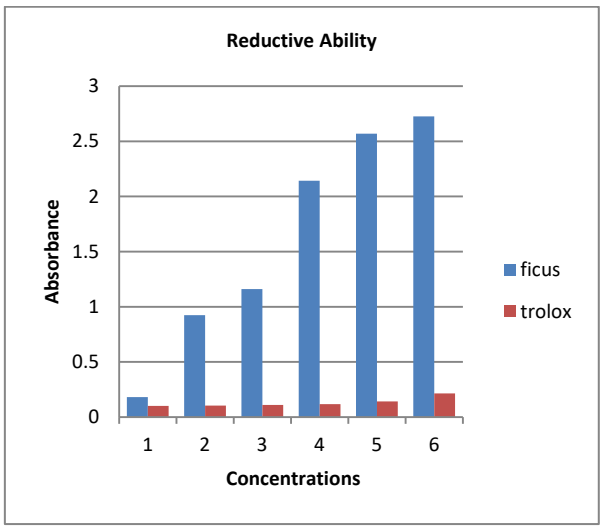
Reductive Ability

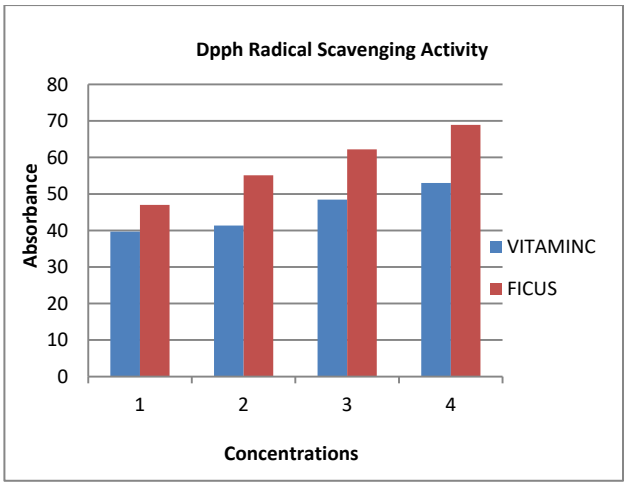
In all concentration tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), the absorbance of *Ficus religiosa* methanolic extract was significantly higher than trolox (vitamin E), and such findings suggest that the plant extract is more effective than trolox in the reductive ability. It was 0.818 ± 0.020 at the concentration 0.02 mg/ml of the methanol extract, and increased significantly to 2.726± 0.030 at the concentration 0.64 mg/ml.

Radical Scavenging Activity of DPPH

Ficus religiosa leaf methanolic extract was significantly more effective in DPPH radical scavenging activity than vitamin C at the four concentrations tested (0.0625, 0.125, 0.250 and 0.500 mg/ml). The concentrations 0.250 and 0.500 mg/ml of plant extract shared an approximated higher radical scavenging activity (62.00 ± 2.00 and 68.00 ± 1.00 %, respectively). Vitamin C also showed variations

between the four concentrations but the difference was not significant.





Antibacterial activity

Results reveals that methanolic extract at lower concentrations(200) mg/ml have moderate antibacterial activity against pathogenic bacteria (*Staph aureus and streptpyogens*) in which the diameter of zone of inhibition range from (15-16)mm for *Streptpyogens* and (14-15)mm for the *Staph aureus,* also increased the concentrations of plant extract lead to increased the diameter of zone of inhibition at the higher concentration (300 and 400)mg/ml to range from (17-22)mm for *Streptpyogens* and from (16-19) for *Staph aureus* shown in figure .

Also study antibacterial activity on other pathogenic bacteria *Pseudomonas aeruginosa* and *Enterobacter ssp* reveals that it was resistant to the concentration (200,300)mg/ml of plant extract while it's have moderate zone of inhibition at high concentration (400)mg/ml in which zone of inhibition range from (13-14)mm for *Pseudomonas aeruginosa* and from (11-13)mm for *Enterobacter spp* as shown in table. While *Escherichia coli* were resistant to the leaves water extract at all concentration.

Table: Antimicrobial activity of *F. religiosa* methanolic extract on growth of *streptpyogens*, *Staph aureus*, *pseudomonas aeruginosa Enterobacter spp* and *Escherichia coli* isolates.

	Concentration of *F.religiosa* water extract (mg/ml) Zone of inhibition (mm)			
Bacteria *Spp*	**200 mg/ml**	**300 mg/ml**	**400 mg/ml**	**Negative control**
Str	16	17	19	R
Str2	15	18	22	R
Str3	16	18	21	R
Sta1	15	16	18	R
Sta2	15	17	19	R
Sta3	14	16	19	R
PS1	R	R	14	R
PS2	R	R	13	R
PS3	R	R	14	R
En1	R	R	12	R
En2	R	R	11	R
En3	R	R	13	R
E1	R	R	R	R
E2	R	R	R	R
E3	R	R	R	R

Str: *Streptpyogens* ;Sta: *Staph aureus* ; PS: *Pseudomonas aeruginosa* ;En: *Enterobacterspp*; E1: *Escherichia coli*

DISCUSSION

The research founded that the major flavonoids in *Ficus* are quercetin and luteolin, with a total of 631 and 681 mg/kg extract, respectively (Bushra and Farooq, 2008). Flavonoids including apigenin, luteolin and quercetin had the ability to inhibit NO production through down regulating iNOS induction (Kim *et al*., 2004). Further studies revealed that natural products such as flavonoids and phenolics have been observed to be efficient free radical scavengers and lipid peroxidation inhibitors (Galleano *et al*., 2010), and probably, the best described and most useful property of almost every group of flavonoids is their capacity to act as antioxidants; protecting the body against reactive oxygen species (ROS) (Xiao *et al*., 2014), The *F. religiosa* leaf methanolic extract inhibits the production of nitric oxide (NO) and proinflammatory cytokines in lipopolysaccharide (LPS) (Frank-Cannon *et al*., 2009) which encouraged microglia via the mitogen activation protein kinase (MAPK) pathway by using different assay including (cell viability, nitric oxide, and enzyme-linked immunosorbent (ELISA)) (Hossain *et al*., 2011) and because *Ficus religiosa* is rich in flavonoid so it acts as antioxidant. The increasing in the reductive ability of *F.religosa* and DPPH radical scavenging activity in comparison with controls of each one (vitamins E and C, respectively) can be attributed to its

high flavonoid content (Hsu *et al*., 2007). All the species of *F. religosa* presented a high antioxidant activity and DPPH radical scavenging activity (Berber *et al*., 2014), which was probably due to the presence of ortho-dihydroxyl of the B-ring (3', 4'-di OH) of the flavonoid molecule which converses high stability to the flavonoid phenoxy radical, C2-C3 double bond that conjugated with 4-oxo group of the ring C participates in radical stabilization by electron delocalization over all three ring system(Chandrasekar *et al*., 2010). The presence of both 3- and 5- hydroxyl moiety of the rings C and A, play an important role in radical scavenging activity of the flavonoids (Zhang, 2013;El-dib *et al*., 2014).

The antibacterial effect of the aqueous and alchoholic extracts of *F. religiosa* was investigated against *Bacillus subtilis, Pseudomonas aeruginosa, E. coli* and *Salmonella typhi*. In an *in vitro* study, (Kamra *et al*., 2008), the ethanolic, methanolic and aqueous leaf extracts of *F. religiosa* exhibited inhibitory effect on methanogensis caused by methanogens (methane producing microorganisms) (Preethi*et al*., 2010;). The effect of ethanolic leaf extract of *F. religiosa* on the growth of different bacterial strains (*Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi, Staphylococcus typhi, Escherichia coli, Shigella dysenteriae* and *Pseudomonas aeruginosa*) were studied by Aqil and Ahmad, 2003, while, Prabhu *et al*., 2009 were studied the effects of plant on filamentous fungi (*Aspergillum niger, Alternaria alternata, Fusarium chlamydosporum, Rhizoctonia bataticola* and *Trichoderma viride*) and yeast (*Candida albicans*). The extract inhibited the growth of *Staphylococcus aureus, Salmonella paratyphi, Shigella dysenteriae, Salmonella typhimurium, E. coli, Salmonella typhi*and *Candida albicans* with zone of inhibition (Nautiyal *et al*., 2007). All the results obtained indicated that the crude extracts and isolated components obtained from *F. religiosa* have shown a good antimicrobial effect against some bacterial and fungal strains (Negi, 2012).

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