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Antioxidant, anti-inflammatory, and antimicrobial evaluation of *Terminalia arjuna* leaves, fruits, and bark

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Abstract: The purpose of this study is to compare the biological activities and the phytochemical contents of *Terminalia arjuna*'s different parts (leaves, fruits, and bark). Firstly, phytoconstituents (tannins, phenols, and flavonoids) in the methanol extracts were determined qualitatively and quantitatively. Followingly, the antioxidant, anti-inflammatory, and antimicrobial capabilities of *T. arjuna*'s leaves, fruits, and bark were evaluated. The antioxidant activity was measured using four assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, hydrogen peroxide (H_2O_2) scavenging, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC), while the anti-inflammatory activity was determined using Human-RBCs membrane stabilisation assay. The antimicrobial potential and MIC were measured against eight selected microorganisms using the agar well diffusion assay and the agar dilution method, respectively. All extracts demonstrated strong antioxidant and anti-inflammatory characteristics. Leaves methanol extract exhibited the highest antioxidant activity with IC_{50} (for DPPH and H_2O_2), FRAP and TAC values of 12.20 µg/mL, 20.60 µg/mL, 3.21 mMol Fe^{+2}/g and 62.74 mg GAE/g respectively. The highest protection against HRBCs lysis was 95.71% for 5 µg/mL dose of leaves methanol extract and 98.22% for 10 µg/mL dose of fruit methanol extract. The antimicrobial activities of the extracts differed clearly. The maximum activity of leaves, fruits, and bark methanol extracts was against *Bacillus subtilis* (MIC=39.06 µg /mL), *Klebsiella pneumonia* (MIC= 156,25 µg /mL), and *Proteus vulgaris* (MIC = 39.06 µg /mL), respectively. These results suggest that *T. arjuna*'s leaves have more potential beneficial effects than bark and fruits, but future *in-vivo* and clinical research is required.

Keywords: *Terminalia arjuna*; Antioxidant; Anti-inflammatory; Antimicrobial; Total phenolics; Total flavonoids; Total tannins.

1. INTRODUCTION

Infectious and chronic diseases are common among the African continent's population. Their treatment and follow-up form a great financial burden [1].

One of the most common causes of inflammations, that are associated with chronic diseases, is oxidative stress. Oxidative stress results from an imbalance in the production and cumulation of reactive oxygen species (ROS) and the protection of the antioxidant system [2]. This imbalance can trigger the inflammation process through various pathways such as direct cytotoxicity, a sensitizing or preconditioning effect to a subsequent insult, or participation in the production of pro-inflammatory mediators [3]. Other stimuli for the synthesis and secretion of proinflammatory mediators are microbial products as bacterial lipopolysaccharides[4]. As a result, if microbial infections and/or free radicals are not controlled, the acute inflammatory state caused by them can progress to chronic inflammation, which may be linked to a variety of chronic diseases.

The WHO global report on traditional and complementary medicine, published in 2019,

declared the presence of a considerable number of Africans depend on complementary, traditional, and alternative medicine as an essential source of primary healthcare [5, 6].

Herbal medicine may provide an effective alternative source of antibiotics, anti-inflammatory drugs, and antioxidants. Moreover, it may have a significant influence on a variety of diseases. Many people prefer the use of herbal drugs to conventional medicine, as they are inexpensive, effective, and safe agents. Several biologically active compounds from natural sources, mainly plants, have been discovered as new medicinal drugs[7]. Consequently, screening the plants, by comparing their different parts, is critical to verify their traditional use, determine the most effective part, and identify the phytoconstituents responsible for the activity.

Natural polyphenols, a group of secondary metabolites present in all plants in varying concentrations, are divided into different classes as flavonoids, tannins, lignans, stilbenes, and phenolic acids[8]. Polyphenols possess various biological activities including prevention and treatment of diseases caused by ROS. So, they are considered powerful antioxidants. These polyphenolic compounds can reduce oxidative stress levels by scavenging free radicals and restricting their synthesis through blocking enzymes or chelating trace elements involved in the production of these radicals. Also, many studies proved the anti-inflammatory activity of polyphenols both *in-vitro* and *in vivo* [9]. Plants' polyphenols can reduce inflammation biomarkers and proinflammatory cytokines as well as increase the synthesis of anti-inflammatory mediators [10]. Furthermore, these phenolics demonstrated a positive impact on bacterial, fungal, and viral infections. These antimicrobial effects of polyphenols resulted from the reduction of a variety of microbial pathogenicity characteristics that include suppression of biofilm formation, neutralization of bacterial toxins and synergism with antibiotics [11].

Terminalia arjuna, a Combretaceae family member, is an Indian native plant that was introduced into Africa a long time ago [12]. Its bark has gained prominence in traditional medicine as a demulcent, astringent, expectorant, antiseptic, heart tonic, antacid, and a remedy for headaches, scorpion stings and snake bites [13, 14]. Reported studies conducted on *T. arjuna* bark have proved its anti-inflammatory, antimicrobial, antioxidant, anticancer, antimutagenic, antilipidemic, antidiabetic, anti-ischemic, and cardiovascular activities[15]. Scientific research has been concerned widely with *T. arjuna* bark ranging from studying its pharmacological activities to isolation and characterization of its active constituents as well as the authentication and quality assessments [13, 14, 16-18]. Furthermore, some clinical studies have been conducted on the bark of *T. arjuna*[16, 19]. On the other hand, there have been few studies concerning *T. arjuna* leaves and fruits.

As a result, this study's main goal was to estimate some pharmacological (antioxidant, anti-inflammatory, and antimicrobial) activities of *T. arjuna* leaves and fruits relative to the bark. Similarly, the phytochemical profiles (tannins, phenolics, and flavonoids) of *T. arjuna* fruits, leaves, and bark were evaluated qualitatively and quantitatively to correlate the phytochemical content and the biological activities.

2. MATERIALS AND METHODS

2.1. Plant Material

Terminalia arjuna (Roxb. ex DC.) Wight & Arn. fruits, leaves and bark were collected in October (2019) from Qanatir, Qalyubia Governorate, Egypt. The plant samples were validated by Dr. Teresa Labib, a plant taxonomist at Orman Botanical Garden, Giza, Egypt. Subsequently, the plant material was air-dried, and voucher specimens [TAL1019, TAF1019, and TAB1019] were maintained in Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University. The dried fruits, leaves and bark were ground and stored in tightly sealed containers till the extraction.

2.2. Extraction

Each powdered plant material (20 g) was macerated in methanol (500 mL) for two days at room temperature three times in a row. Then methanol extracts of fruits (FME), leaves (LME), and bark (BME) were filtered using Whatmann No. 1 filter paper and evaporated under vacuum at 50 C°. The following formula was used to calculate the percentage yield of each extract:

Yield (%) = (W / W*) X 100

Where W = weight of each extract after evaporation and W * = weight of each macerated powder.

2.3. Qualitative Analysis of Phytochemicals

FME, LME and BME were tested for the presence of different phytoconstituents using

standard methods described by Harborne [20] and Khandelwal [21].

2.4. Quantitative Analysis of Phytochemicals

2.4.1. Quantitative Determination of Total Phenolic Content (TPC)

TPC in each of FME, LME and BME was assessed spectrophotometrically using the Folin-Ciocalteu method as described by Singleton *et al* [22].The results were calculated using the gallic acid standard curve and represented as gallic acid equivalents (GAE).

2.4.2. Quantitative Determination of Total Flavonoid Content (TFC)

TFC was measured for each extract via the aluminum chloride colorimetric assay explained by Zhishen *et al* [23]. Flavonoid concentrations were calculated using a quercetin calibration curve and represented as quercetin equivalents (QE).

2.4.3. Quantitative Determination of Condensed Tannin Content (CTC)

Vanillin assay described by Sun *et al.* [24] was used for measuring CTC in each of FME, LME and BME. CTC was quantified from the catechin standard curve and expressed as catechin equivalents (CE).

2.5. *In-vitro* Evaluation of Antioxidant Activity

Various antioxidant techniques were applied to evaluate the antioxidant capacity of FME, LME, and BME. Both ascorbic acid and BHT (butylated hydroxytoluene) were used as standard references in all assays.

2.5.1. DPPH Free Radical Scavenging Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of each extract was measured according to Yen and Duh [25]. The following formula was used to calculate the percentage inhibition (PI) of the DPPH radicals:

$$\text{PI} = \frac{A_C - A_E}{A_C} \text{ x } 100$$

Where A_C= absorbance of the control (DPPH + methanol) and A_E = absorbance of the extract. The IC_{50} value denotes the extract concentration necessary to scavenge DPPH free radicals by 50%.

2.5.2. Hydrogen Peroxide (H_2O_2) Scavenging Assay

The ability of each extract to scavenge H_2O_2 was determined as described by Ruch *et al* [26]. The following equation was used to calculate the percentage scavenging of H_2O_2 by each extract:

$$H_2O_2 \text{ scavenging (\%)} = \frac{A_C - A_E}{A_C} \times 100$$

Where A_C= Absorbance of the control (H_2O_2 + methanol) and A_E= absorbance of the extract. The IC_{50} value represents the extract concentration required to scavenge H_2O_2 by 50%.

2.5.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of each extract was evaluated according to Benzie and Strain [27]. This method utilizes the ability of the extract to reduce ferric to ferrous ions as an indication of its potential antioxidant property. Then the result was determined against a standard curve of ferrous sulphate and expressed as mMol Fe^{+2}/g dry extract.

2.5.4 Total Antioxidant Capacity (TAC) Assay

TAC of each extract (FME, LME, and BME) was also evaluated spectrophotometrically by the phosphomolybdate complex method described by Prieto *et al* [28]. The antioxidant capacity was calculated using the gallic acid standard curve and expressed as mg gallic acid equivalent (GAE) per gram dry extract.

2.6. *In-vitro* Evaluation of Anti-Inflammatory Activity

The *in-vitro* anti-inflammatory activity of FME, LME and BME was estimated using human red blood cells (HRBCs) membrane stabilization assay as explained by Gandhidasan *et al* [29]. Both diclofenac sodium and aspirin were used as standard drugs, and the anti-inflammatory activity was expressed as the percentage of protection from RBC lysis. The percentage of blood hemolysis was calculated using the following formula:

$$\%\ Hemolysis = \left(\frac{Extract\ optical\ density}{Control\ optical\ density}\right) X\ 100$$

The following formula was used to calculate the percentage of human RBCs membrane protection or stabilization:

$$\%\ Protection = 100 - \left(\frac{Extract\ optical\ density}{Control\ optical\ density}\ X\ 100\right)$$

2.7. Evaluation of Antimicrobial Activity

The agar well diffusion method described by Hindler et al.[30] was used to evaluate the antimicrobial activities of FME, LME and BME. To make the inoculum suspension, colonies were cultivated overnight on an agar plate and then inoculated into Mueller-Hinton broth (malt broth was used for fungi). Consequently, a sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (malt agar plates were used for fungi). An aseptically punched hole with a diameter of 6 mm was made. Each extract dissolved in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/mL was introduced into the well. The diameter of the inhibition zone (IZ) around each well was measured after 24h at 37 C^{o} and compared the results with those of positive control using gentamycin (4μg/mL) for bacteria and ketoconazole (100 μg/mL) for fungi. For determination of the minimum inhibitory concentration (MIC), the agar dilution method was followed. In which two-fold serial dilutions of FME, LME and BME were prepared. Each dilution of different extracts is combined with a molten agar medium to produce the plates. Into these plates, a standardized number of bacteria or fungi is inoculated. Finally, the plates were incubated for 24 h at 37 C^{o}, and MIC (the least dilution inhibits the visible growth) was determined.

2.8. Statistical Analysis

Linear regression analysis approach was used for determination of TPC, TFC, CTC, FRAP, TAC and IC_{50}. All samples were analysed three times, and the results are reported as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The present work estimated the chemical content, antioxidant activity, HRBCs lysis protection, and antimicrobial activity of *T. arjuna* fruits, leaves and bark. The purpose is to assess the extent to which fruits and leaves can be utilized in therapy by comparing their efficacy to that of the bark, which is a well-known antioxidant, anti-inflammatory, and antibacterial agent.

3.1. Qualitative Analysis of Phytochemicals

Different phytoconstituents were determined in each of FME, LME and BME according to standard phytochemical tests. The preliminary phytochemical screening proved the presence of the same classes of active constituents in all extracts, as shown in Table (1). The results agree with previous studies [31-33].

3.2. The Yield and Quantification of TPC, TFC and CTC

Using methanol as solvent, each of T. arjuna fruits, bark, and leaves produced roughly the same yield. The extract yields were 17%, 18% and 20% (w/w) respectively. FME, LME and BME all have a considerable quantity of phenols and tannins and to a lesser extent flavonoids are present. TPC, TFC and CTC were represented as mg GAE, QE or CE/ g extract respectively. The highest concentration of phenolic constituents and tannins was found in BME while the highest flavonoid content was found in LME. Results are presented in Table (2), and they differ to some extent from concentrations published in the literature. According to previous studies, T. arjuna grown in different areas of the same country exhibited significant variations in the concentration of phenols, flavonoids, and tannins in its different parts. [34-36]. These variations indicate the role of the environmental factors in affecting the phytochemical composition of the same plant [34]. So, variations in the quantification of components of a plant grown in different countries are not only prevalent, but also necessary for standardization and quality control in order to determine the plant's origin.

Table 1. Qualitative analysis of phytoconstituents present in *T. arjuna* FME, LME, and BME.

Phytoconstituent	FME	LME	BME
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Anthraquinones	–	–	–
Alkaloids	+	+	+
Carbohydrates and/or glycosides	+	+	+
Terpenoids	+	+	+

"+" means present, while "-" means absent.

Table 2. Total phenol, tannin, and flavonoid contents in *T. arjuna* FME, LME, and BME.

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	CTC (mg CE/g)
FME	137.98 ± 10.64	19.51 ± 1.37	53.76 ± 1.92
LME	96.42 ± 3.86	23.65 ± 1.97	64.89 ± 3.53
BME	184.59 ± 13.25	22.98 ± 1.46	118.73 ± 9.21

3.3. *In-vitro* Antioxidant Activity

The antioxidant activity of FME, LME, and BME was assessed using various established *in-vitro* techniques because a single antioxidant assay cannot characterize the whole antioxidant activity of any extract. The DPPH, H_2O_2, FRAP, and TAC assays were used to acquire quantitative data on the antioxidant activity of the extracts due to their difference in many aspects as a mode of action, targets, time and temperature, pH, and the use of standards to produce analytical curves. [37].

DPPH, a stable free radical with deep purple colour, is neutralized by receiving either an electron or a hydrogen atom from an antioxidant species. This results in gradual decolorization of DPPH colour to pale yellow. The more the colour of DPPH disappears, the better the antioxidants potential to scavenge free radicals. This assay is simple, sensitive, cheap, and rapid. It is used mainly for quantifying antioxidants with low activity or in complicated systems. The main limitation of this assay is the lack of DPPH radicals in the human body, which results in a non-physiological similarity to the free radicals [38].

Hydrogen peroxide can be formed *in vivo* by metabolism process and cross cell membrane to attack many cellular compounds. By itself, H_2O_2 is not a free radical but it is ready to convert to the indiscriminately reactive hydroxyl radical ($OH^{\bullet}$) by interaction with a range of transition metal ions [39]. In this assay, the absorbance of the reaction mixture contains extract decreased as compared to control. This method is characterized by its ease, sensitivity and specificity but it is pH sensitive [38].

FRAP assay is used to estimate the extracts' reducing power. In which ferricyanide accepts an electron from the antioxidant analyte to be converted into the reduced form of ferrocyanide. Consequently, it is changed from yellow to Prussian blue in the presence of ferric chloride. In contrast to DPPH and H_2O_2 assays, this method depends on the principle of increasing the absorbance of reaction mixture. Increased absorbance means higher redox potential of the extract [40].

The TAC (phosphomolybdenum) assay was first used to determine the amount of vitamin E in seeds. Its applicability has been extended to plant extracts due to its simplicity and sensitivity. In this assay, the reduction of molybdenum (VI) to molybdenum (V) occurs via either electron transfer or hydrogen atom transfer. As the reaction colour shifted from yellow to greenish blue, the antioxidant activity of the extract increased by increasing its absorbance. One of the key benefits of TAC assay is the capability to screen a broad range of samples involving lipophilic plant extracts. However, the assay demonstrated a bad correlation with flavonoids and phenolics content due to its ability to detect other compounds as ascorbic acid, carotenoids and α-tocopherol[38].

As shown in Tables (3) and (4), the current study revealed that all extracts had considerable antioxidant activities that were proportionate to concentration. LME possessed the highest antioxidant activity compared to both BME and FME although it had the lowest phenolic content. LME inhibited DPPH and H_2O_2 in a dose-dependent manner with the highest inhibition percent of (97.03 ± 0.29 and 96.29 ±0.73) and IC_{50} of (12.2 ± 1.12 and 20.6 ± 1.86) respectively. Also, LME had the highest value of FRAP (3.21± 0.65 mMol Fe^{+2}/g) which exceed the reference standard ascorbic acid (2.93 ± 0.75 mMol Fe^{+2}/g). Finally, LME exhibited the highest antioxidant capacity equivalent to 62.74 ± 3.42 mg of gallic acid/g in TAC assay. This potent antioxidant activity of LME which exceeds both BME and FME, may be attributed not only to its phenolic components but also to non- phenolic constituents. These findings were consistent with prior research on the ability of plant extracts' non-phenolic content to contribute to scavenging and antioxidant effects [41, 42].

Table 3. Antioxidant activities of *T. arjuna* FME, LME, and BME via DPPH, H_2O_2, FRAP and TAC assays.

Sample	DPPH [a]IC_{50} (µg/mL)	H_2O_2 [b]IC_{50} (µg/mL)	FRAP (mMol Fe^{+2}/g)	TAC mg GAE/g
FME	16.9 ± 1.36	30 ± 2.17	1.94 ± 0.62	60.92 ± 3.96
LME	12.2 ± 1.12	20.6 ± 1.86	3.21 ± 0.65	61.74 ± 3.42
BME	13.3 ± 1.07	33.1 ± 1.94	2.54 ± 0.48	53.27 ± 4.39
Ascorbic acid	10.6 ± 0.8	12.74 ± 0.86	2.93 ± 0.75	124.83 ± 7.21
[c]BHT	8.24 ± 0.62	12.3 ± 0.7	6.84 ± 0.92	76.91 ± 4.53

a and b = the concentrations of extracts that scavenge DPPH radicals and H_2O_2 by 50% respectively.,
c = standard reference butylated hydroxytoluene.

Table 4. Percentage scavenging activity of *T. arjuna* FME, LME, and BME on DPPH radicals and H_2O_2.

Conc. (µg/mL)	H_2O_2 Scavenging %			DPPH scavenging %		
	FME	**LME**	**BME**	**FME**	**LME**	**BME**
1280	94.72±0.64	96.29±0.73	93.87±1.21	96.84±0.42	97.03±0.29	96.74±0.28
640	92.96±0.52	93.87±0.29	90.42±0.64	95.48±0.26	96.61±0.35	95.17±0.59
320	90.35±0.19	91.46±0.52	86.08±1.36	93.61±0.73	94.98±0.74	93.40±0.68
160	84.29±0.75	90.54±0.34	81.94±1.08	92.94±0.54	93.72±0.56	92.76±0.92
80	73.26±1.92	85.91±0.75	70.52±2.37	90.37±0.65	92.98±0.64	90.81±1.53
40	59.63±2.31	72.86±1.42	56.45±2.81	81.42±1.74	91.74±1.08	87.68±0.94
20	40.35±2.49	49.28±2.76	37.62±2.46	62.04±2.32	73.93±2.85	58.04±2.68
10	19.47±1.37	31.72±1.59	22.64±1.24	23.68±1.94	43.16±1.82	45.95±2.31
5	9.82±0.54	24.65±0.93	7.21±0.63	11.75±0.23	30.27±0.71	29.73±0.95
2.5	3.69±0.35	12.84±0.62	4.93±0.25	4.21±0.35	23.85±0.93	18.65±0.47
0	0.00	0.00	0.00	0.00	0.00	0.00

3.4. *In-vitro* Anti-Inflammatory Activity

Stabilization of HRBCs membrane exposed to hypotonicity was used to evaluate the anti-inflammatory activity of FME, LME and BME. This method was preferred over other *in-vitro* assays due to its ease of use and reliability. Hypotonicity caused by excessive fluid accumulation inside the RBCs membrane leads to its rupture and lysis. Because both erythrocyte and lysosomal membranes are similar, RBCs membrane stabilization implies lysosomal membrane stability. As a result, the discharge of hydrolytic lysosomal enzymes and other active inflammatory mediators as well as additional damage and inflammation of tissue is inhibited [43]. According to the results reported in Table (5), all extracts exerted substantial HRBCs stabilizing effects which were almost like or greater than compared reference standards (aspirin and diclofenac). Each FME, LME and BME prevented haemolysis in a concentration-dependent manner. At the lower concentration (0.005 mg/mL), LME provided the best protection against haemolysis (95.71%), while FME provided the best protection (98.22%) at the higher concentration (0.01 mg/mL). Both aspirin and diclofenac (0.01 mg/mL) demonstrated an inhibition percentage of 97.92% and 97.84% against haemolysis, respectively. Finally, the *in-vitro* results suggested that the methanol extract of *T. arjuna* leaves and fruits had a stronger anti-inflammatory effect than the methanol extract of the

bark. This potent inhibitory effect of HRBCs lysis may refer to the high content of saponins, tannins and flavonoids in the extracts. As reported in the previous studies that flavonoids and some saponins have a significant effect on the stability of lysosomal membranes both *in vivo* and *in-vitro*, whereas saponins and tannins stabilize erythrocyte membranes with their potential to bind to cations [44-46].

3.5. **Antimicrobial Activity**

The antimicrobial properties of FME, LME and BME were screened against gram-positive and gram-negative bacteria as well as fungi using the agar well diffusion method. The results revealed that all extracts in a concentration of 20 mg/mL could inhibit the growth of all tested microorganisms. As stated in Table (6), LME has the maximum zone of inhibition against each of *Candida albicans* (fungi), *Streptococcus mutans* and *Bacillus subtilis* (gram-positive bacteria). BME showed maximum inhibition zones against two gram-positive bacteria: *Bacillus subtilis* (the same IZ as LME) and *Staphylococcus aureus*. FME demonstrated the largest IZ against *Aspergillus fumigatus* (fungi) and the gram-positive bacteria *Staphylococcus aureus* (=IZ of BME) as well as all tested gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris*. Significant antimicrobial effects, expressed as MIC, revealed variability in their values among extracts. The lowest MIC values (39.06 and 78.13 µg/mL) were exhibited by LME and BME. LME exhibited MIC of 39.06 µg/mL against *Bacillus subtilis* and of 78.13 µg/mL against *Proteus vulgaris* while BME showed MIC of 39.06 µg/mL against *Proteus Vulgaris* and of 78.13 µg/mL against both *Escherichia coli* and *Streptococcus mutans.*

Table 5. Effects of *T. arjuna* FME, LME, and BME on human RBCs membrane stabilization.

Sample	% Hemolysis	% Protection
FME: (0.005mg)	7.92 ± 0.21	92.08 ± 0.23
(0.01mg)	1.78 ± 0.21	98.22 ± 0.23
LME: (0.005mg)	4.29 ± 0.12	95.71 ± 0.21
(0.01mg)	3.94 ± 0.12	96.06 ± 0.21
BME: (0.005mg)	5.36 ± 0.11	94.64 ± 0.15
(0.01mg)	5.12 ± 0.11	94.88 ± 0.15
Aspirin (0.01mg)	2.08 ± 0.09	97.92 ± 0.09
Diclofenac (0.01mg)	2.16 ± 0.11	97.84 ± 0.11
Distilled water (control)	100.00	0.00

Table 6. Antimicrobial activities of *T. arjuna* FME, LME, and BME by determination of inhibition zone (IZ) and minimum inhibitory concentration (MIC).

Tested microorganisms	FME		LME		BME		Control
	IZ (mm)	MIC (µg/mL)	IZ (mm)	MIC (µg /mL)	IZ (mm)	MIC (µg /mL)	IZ (mm)
Fungi							Ketoconazole
Aspergillus fumigatus	10	5000	9	5000	8	10000	17
Candida albicans	8	10000	10	10000	9	10000	20
Gram positive bacteria							Gentamycin
Staphylococcus aureus	9	10000	8	5000	9	10000	24
Streptococcus mutans	17	625	20	156.25	15	78.13	22
Bacillus subtilis	13	1250	20	39.06	20	156.25	26
Gram negative bacteria							Gentamycin
Escherichia coli	20	156.25	18	312.5	19	78.13	30
Klebsiella pneumonia	20	156.25	15	625	18	312.5	21
Proteus vulgaris	18	312.5	17	78.13	15	39.06	25

5. CONCLUSIONS

Natural polyphenols are currently a prominent focus of research since they are thought to be effective chain-breaking antioxidants, antibacterial, anti-inflammatory, anticancer, and antiviral therapies. In the current study, phenolic, flavonoid and tannins contents as well as antioxidant, antimicrobial, and anti-inflammatory activities were compared in the methanol extracts of *T. arjuna* fruits, leaves, and bark. Leaves methanol extract showed the highest DPPH free radicals and hydrogen peroxide scavenging activities as well as the greatest ferric reducing antioxidant power and total antioxidant capacity. The leaves extract also exhibited the maximum stabilization for HRBCs membrane at concentration of 0.005 mg/mL and the best antimicrobial activity mainly against *Bacillus subtilis*. According to the findings of this study, *T. arjuna* leaves methanol extract can be employed as a renewable supply of natural safe antioxidants and anti-inflammatory in dietary supplements or nutraceutical formulations, however additional studies are recommended.

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Author Contribution: Fatma Abo-Elghiet: study design and manuscript writing., Alaa Abd-elsttar: carrying out the practical work., Ahmad M. Metwaly: study design, supervision, and manuscript revision., Abd-Elsalam I. Mohammad: Supervision.

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