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In-vitro Antimicrobial Potentialities of Phylunthus emblica Leaf Extract Against Some Human Pathogens

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Abstract

Phyllanthus emblica is used in traditional medicine due to its bioactive constituents. This study aimed to evaluate the antimicrobial activity of the *Phyllanthus emblica* methanol extract and its chemical profile. The plant was extracted with 85% methanol and the total phenolic and flavonoid contents as well as antimicrobial activity on some Gram- positive, Gram-negative bacteria and some fungi species were evaluated. Also, GC-MS analysis of plant extract was administered. *Phyllanthus emblica* methanolic extract showed high total phenolic content (216.41± 2.1 mg GAE/g of dry extract) and total flavonoid content (32.31± 1.2 mg RE/g of dry extract). The antimicrobial activity of extract against some pathogenic microbes proved that the plant extract is effective against all species except *Escherichia coli*. The highest activity was showed against *Aspergillus fumigatus* & *Syncephalastrum racemosum* (fungi) as well as *Staphylococcus aureus*, *Syncephalastrum racemosum* & *Streptococcus pneumonia* (bacteria). GC-MS analysis of *Phyllanthus emblica* exhibited the presence of thirty-one bioactive compound. The major compounds are 1, 3-Dimethylindole (24.11%), Isopropyl palmitate (23.94%), Gamolenic acid (7.73%) 1, 5-Hexanediol (7.58%), 8, 11-Octadecadienoic acid methyl ester (6.66%), 2-Methylhexadecanoic acid (5.62%) and Linolenic acid (3.49%). Thus, *Phyllanthus emblica* may be used as a potential oral antibiotic against some pathogenic microbes.

Keywords: GC-MS analysis; Flavonoid content; Phenolic content; Phytochemical screening

1. Introduction

Herbs have been the origin of many traditional medical schemes throughout the world for thousands of years and still provide human with new therapies. World Health Organization (WHO) expected that 80% of the developing countries populations still depend on plant drugs in traditional medicines due to their safety. Also, modern pharmacopoeia contains at least 25% drugs derived from plants[1,2]. Natural drugs from plant sources have a great economic value when compared with artificial ones from their cost and side effects. The role of plants is not involved only in therapeutic, but also in maintaining good health[3,4]. *Phyllanthus emblica* L. belongs to the family Euphorbiaceae[5]. The plant is usually referred to as Amlaki[6]. The leaves, roots and bark of the plant are used for the treatment of dysentery and warts. The leaves of *P. emblica* have been used in aphrodisiac, useful in nausea, asthma, bronchitis, leucorrhoea and vomiting. Also, fruits of *P. emblica* have been reported with their antipyretic and anti-inflammatory properties as well as they have been used as a tonic during winter in India[6]. Human pathogens are varied and widely diverse involved unicellular and multicellular eukaryotes besides viruses and bacteria that belong to billions of microbial species as human pathogens[7]. Microbes are dangerous microorganisms (fungi and bacteria). The infections of them have increased effectively, especially in immune system disorders. The ability of medicinal plants to stop the growth of microbes is due to the chemical compounds in these plants that possess antimicrobial activity[3]. Gas chromatography-mass spectrometry (GC-MS) is a powerful technique as it is specific and highly sensitive. The specific detection and identification of compounds by GC-MS depend on the molecular mass in a mixture. Also, the combination of a

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principle separation technique (GC) with the best identification technique (MS) made this technique perfect for qualitative and quantitative analysis especially for volatile and semi-volatile compounds[8]. Our present study aimed to identify the chemical constituents of *Phylanthus emblica* methanolic extract by GC-MS analysis and evaluating its antimicrobial activity.

2. Experimental

Collection of plant

The plant was collected from Qnater gardens, Cairo, Egypt, early in the morning in October 2019 in the ripening stage of the fruits. It was identified by a senior plant taxonomist, Dr Abd El Halim Mohamed, Flora and Taxonomy Department, Agricultural Research Center Dokki, Giza. The voucher specimen was preserved within the laboratory of the Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, Egypt.

Extraction of plant

The leaves of the plant were dried and powdered by a grinder. 500g of dried plant leaves was soaked in 85% methanol for 1 week. Then, they were evaporated under vacuum using a rotatory evaporator till dryness. The crude extract was kept away from moisture to use in the present study.

Phytochemical Screening

Phytochemical screening of the methanol extract of *Phylanthus emblica was* carried out according to El-Sayed *et al* [9] and Fitriansyah *et al* [10]. Phenols and tannins were detected by $FeCl_3$ 10%, Alkaloids were detected by Mayer's and Wagner's tests. Carbohydrates were detected by Molisch's test. Also, Shinoda test was used for flavonoids while, froth test was for saponins. Libermann-Berchard test is used for steroids and triterpenoids, 10% vanillin in H_2SO_4 was used for monoterpines and sesquiterpines and Borntrager's test for quinons.

Total phenolic content

The phenolic content of the tested extracts decided employing a spectrophotometric method described by Abdel-Hady *et al.*[11]. Using Foline-Ciocalteus and measured at wavelength 765nm. 0.5ml of plant extract (250µg/ml); 2.5ml of Folin-Ciocalteus reagent (10%) dissolved in water and 2.5ml $NaHCO_3$ (7.5%). Blank sample contains 0.5ml MeOH, 2.5ml of Folin-Ciocalteus reagent (10%) dissolved in water and 2.5ml $NaHCO_3$ (7.5%). The mixtures were shaken and incubated at 45°C for 45min. The absorbance was recorded against a blank sample; gallic acid was used as the standard. The experiment was carried out in triplicate. The total phenolic content was expressed in terms of gallic acid equivalent (GAE) per gram dry weight of the extract.

Total Flavonoid content

Determination of total flavonoid content was determined according to Abdel-Hady *et al.*[12] Using a colorimetric assay and measured at wavelength 510nm. 0.5ml of plant extract was mixed with 2ml distilled water and 150µl of $NaNO_2$ (5%) for 6 min, then 150µl of $AlCl_3$ (10%) was added and allow to stand for 5min, then added of 2ml NaOH (4%) and adjusted to 5ml with 200µl distilled water. The mixtures were incubated at room temperature for 15min. the absorbance was measured against a blank sample; rutin was used as the standard. The experiment was carried out in triplicate. The total flavonoid content was expressed in term of mg rutin equivalents (RE) per gram extract.

Antimicrobial test

The antimicrobial activity of the extract was determined using the agar well diffusion method [13]. The plant was tested in vitro for its antibacterial activity against *Streptococcus pneumoniae* and *Staphylococcus* aureus (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria) using a nutrient agar medium. Antifungal activity was administered against *Aspergillus fumigatus*, *Syncephalastrum racemosus*, *Geotricum candidum* and *Candida albicans* using the sabouraud dextrose agar medium. Gentamicin, Ampicillin and Amphotricin B were used as standard drugs for gram-positive, gram-negative and antifungal activity, respectively. DMSO was used as solvent control. The concentration of a tested sample and standard drugs was 1mg/ml against both bacterial and fungal strains.

3. Method of testing

20ml of sterilized media was poured into the sterilized Petri-dishes and allowed to solidify. Wells of 6mm diameter were made in the media with the help of a sterile borer. 1ml of every microbial suspension was poured on the surface of the solidified media, and evenly distributed employing a sterile swab. Solution of the tested sample was added to each well with the help of a micropipette. The plates were incubated at 37^0c for 24hrs just in a case

of antibacterial activity while 48hrs at 25^0c for antifungal activity. This experiment was performed in triplicate and zones of inhibition were measured in mm scale.

GC-MS Analysis

The crude methanol extract of the plant was subjected to GC-MS analysis using Thermo Scientific TRACE 1310 Series Gas Chromatograph on Helium as a carrier gas in TG-SQC column [13]. Analysis of the sample was held at temperature program as follows: initial temperature 50^0c for 1min, then increased to 250^0c for 5min, finally increased to 290^0c for 2min. Sample was injected in split mode constant flow 1.5ml/min. Mass spectral range was set as 40-1000Hz also, mass transfer line temperature was 300^0c and ion source temperature was 300^0c then identification of components was carried out by using computer search in data libraries.

4. Results and Discussion

Phytochemical Screening

Phytochemical screening is the first step to screen the compound groups that exist in the plant. Phytochemical screening of *Phylanthus emblica* leaves as shown in (**Table 1**) exhibited the presence of alkaloids, flavonoids, phenolic, carbohydrates, tannins, terpenoids and saponins.

Phenolic and flavonoid compounds cause the antioxidant activity of the plant as well as increase the polarity of compounds[10]. These results are in full agreement with previous studies on *Phylanthus emblica*[14,15,10].

Table 1: Phytochemical screening of *Phyllanthus emblica* methanol extract

Phytochemical conistituents	Methanol extract of leaves
Phenols	+
Tannins	+
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Saponins	+
Steroids	-
Triterpenoids	+
Monoterpines	+
Sesquiterpines	+
Quinons	+

Total Phenolic and Flavonoid Contents:

Phenols and flavonoids are the secondary metabolites that have been known for their antioxidant activity [12]. The antioxidant activity of these secondary metabolites is due to the ability to scavenge reactive free radicals [11]. The methanol extract of *Phylanthus emblica* recorded total phenolic content (216.41± 2.1 mg GAE/g of dry extract) and total flavonoids (32.31± 1.2 mg RE/g of dry extract) as shown in (**Fig 1).** Different species of *Phylanthus emblica* may be varied due to different agricultural environmental and conditions [16]. Our results agreed with many studies on the plant extract [17, 18, 19, 20].

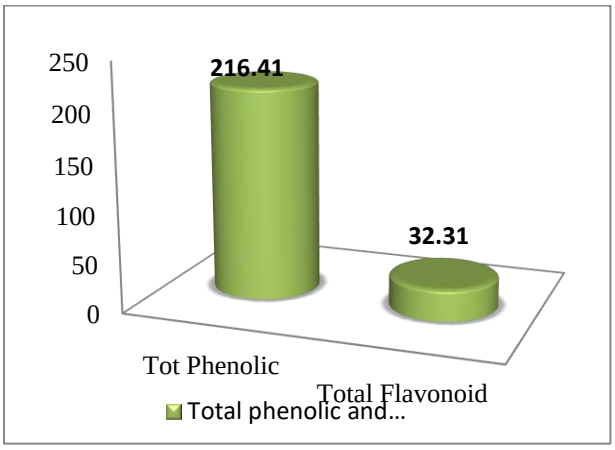


Fig 1: Total phenolic and flavonoid contents of *Phyllanthus emblica* methanol extract

Antimicrobial test

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health [21]. Nowadays, they are more widespread and have been persisted extremely especially in immunodeficiency members. Many bacterial species are resistant to antibiotics while their growth was inhibited by different plant extracts [3].

Phylanthus emblica extract in **Table (2)** showed its antimicrobial activity against some bacteria and fungi species. The results revealed that plant extract has antibacterial activity against *Streptococcus pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* while, there is no antibacterial activity was shown against *Escherichia coli*. Also, the plant extract exhibited antifungal activity against both *Aspergillus fumigatus* and *Syncephalastrum racemosum*. These results agree with other reports that proved the antimicrobial activity of *Phylanthus emblica*. It was reported that fruits of *Phylanthus emblica* showed antimicrobial activity against pathogenic bacteria; *Staphylococcus*

aureus [22]. Also, Nanasombat *et al* [23] proved the activity of fruit extract against *Pseudomonas fluorescens*, *Bacillus cereus* and *Staphylococcus aureus.* While, he did not prove any activity against fungi. Likewise, the methanol extract of the plant proved its antibacterial activity against *Staphylococcus aureus* as well as antifungal activity against *Candida albicans*, *Candida tropicalis* and *Aspergillus niger* [24]. Dhale *et al* [14] reported that different extracts as petroleum ether and chloroform of *Phylanthus emblica* also have antibacterial and antifungal activity. Actually, there are not enough studies carried out on the methanol extract of *Phyllanthus emblica* leaves. As well, few reports of leaf effect were exhibited only on a few pathogen species from the tested ones. So, we cared to demonstrate the antimicrobial activity of leaf on a large scale of pathogens.

GC-MS Analysis

The bioactive components of methanol extract of *P. emblica* were evaluated by GC-MS analysis (**Table 3**, **Fig 2**) showed the presence of thirty-one compounds. The identification of the component of the extract was performed by their retention time (RT), molecular formula (MF), molecular weight (MW), and concentration (%). In which, 14 compounds of them were higher than 1% relative to the total bioactive components of extract of *P. emblica.* The major compounds in the extract identified fatty acids were Isopropyl palmitate (23.94%), Gama-linolenic acid (7.73%), 8, 11-Octadecadienoic acid methyl ester (6.66%), 2-Methylhexadecanoic acid (5.62%) and Linolenic acid (3.49%) which are commonly known to possess antimicrobial, antioxidant, antibacterial and antifungal activities[25,26]. 1, 5-Hexanediol (7.58%) was detected as one of the major components in the methanol extract of *P. emblica*. It has antimicrobial activity against Gram-positive and Gram-negative bacteria and has already been reported as anti-inflammatory and other beneficial activities related to cosmetics[27]. 1, 3-Dimethylindole (24.11%) is an Indole derivative that is classified as aromatic heterocyclic. Indole derivatives have different biological activities, i.e., anti-inflammatory, antiviral, antioxidant, anticancer and antimicrobial [28]. These results revealed that the chemical constituents of the methanol extract of *P. emblica* leaves are in agreement with previous studies reported by [29, 30]

5. Conclusion

This report concerned with the antimicrobial effect of *Phyllanthus emblica* leaf extract where, more antimicrobial studies were carried out on different extracts as petroleum ether, chloroform and ethyl acetate of the plant leaf more than methanol extract. In addition, fruits had more attention than leaf. The results of this study exhibited promising antimicrobial activity of the plant leaf against tested pathogenic bacteria and fungi except *Escherichia coli*. The activity of the plant may be returned to the phenolic and flavonoid contents. That is due to the strong biological activity of these secondary metabolites. Furthermore, GC-MS analysis confirmed the antimicrobial activity of the plant through the presence of some major compounds such as: Isopropyl palmitate, Linolenic acid, 1, 5-Hexanediol and 1, 3-Dimethylindole.

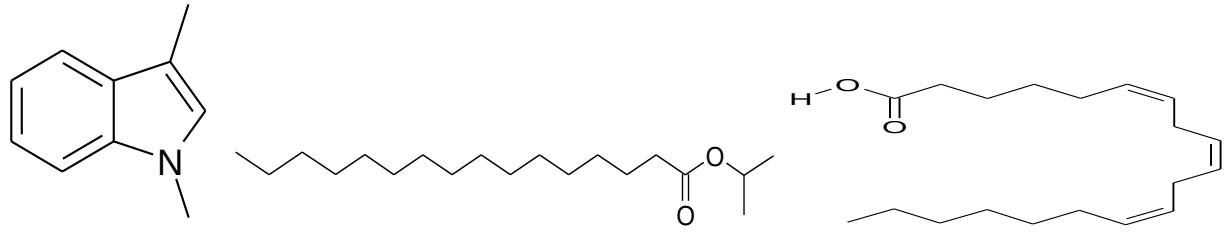
Table 2: Antimicrobial activity (mean zone of inhibition in mm) of *phyllanthus emblica* methanol extract

Tested Microorganisms	*Phyllanthus emblica*	Standard antibiotics
Gram Positive Bacteria		**Ampicilin**
Streptococcus pneumoniae (RCMB 010010)	10 ± 1.2	22.7± 1.3
Staphylococcus aureus (RCMB 000106)	12 ± 1.8	24.1± 1.8
Gram Negative Bacteria		**Gentamicin**
Pseudomonas aeruginosa (RCMB 010043)	11 ±0.9	15.1± 0.38
Escherichia coli (RCMB 010052)	Nil	17.7± 0.42
Fungi		**Amphotericin B**
Aspergillus fumigatus (RCMB 02568)	13 ±1.7	21.7± 0.38
Syncephalastrum racemosum (RCMB 05922)	10 ± 1.2	13.7± 1.7
Geotricum candidum (RCMB 05097)	7 ± 1.1	26.5± 1.2
Candida albicans (RCMB 05036)	8 ± 1.3	23.4± 0.43

*Nil: No activity, RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms.
*Data are expressed in the form of mean ± SD.
*Concentration used for both fungi and standards are 1mg/ml.

Table 3: The GC-MS of Methanol extract of *P. emblica* family Euphorbiaceae

No	RT	Area %	MF	MW	Name	Compound Nature
1	3.50	7.58	C6H14O2	118	1,5-Hexanediol	aliphatic alcohol
2	8.88	0.73	C6H12O6	180	D-Mannose	sugar monomer
3	9.85	24.11	C10H11N	145	1,3-Dimethylindole	aromatic heterocyclic
4	12.19	0.80	C27H44O3	416	24,25-Dihydroxy vitamin D	fat-soluble secosteroid
5	13.20	0.33	C9H11NO4	197	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester)	heterocyclic ester
6	13.26	1.41	C14H22O	206	Phenol,2,4-di- tert-butyl-	phenol
7	13.73	2.02			Menthol,1'-[butyln-3-one-1-yl)-, (1R, 2S, 5R)	
8	15.01	0.90	C18H30O2	278	10-Heptadecen-8-ynoic acid, methyl ester, (E)	Ester
9	15.38	0.43	C36H60O2	524	Vitamin A palmitate	Ester
10	15.51	0.47	C20H38O2	310	Cis-11-Eicosenoic acid	Fatty acid
11	15.81	1.89	C10H18	138	3-Isopropyl-6-methylcyclohexane	
12	15.89	1.57	C18H32O2	280	17-Octadecynoic acid	Fatty acid
13	15.97	0.34	C17H32O2	268	Cis-10-Heptadecenoic acid	Fatty acid
14	16.08	0.35	C18H32O2	280	17-Octadecynoic acid	Fatty acid
15	16.23	0.72	C19H34O2	294	Methyl-cis-9,cis-15-linoleate	linoleate
16	16.32	0.76	C15H26O2	238	Geranyl isovalerate	carboxylic ester
17	16.56	5.62	C17H34O2	270	2-Methylhexadecanoic acid	Fatty acid
18	16.74	0.48	C22H42O2	338	Erucic acid	Fatty acid
19	16.90	23.94	C19H38O2	298	Isopropyl palmitate	Fatty acid ester
20	17.59	0.39	C22H42O3	354	n-Butyl ricinoleate	Fatty acid
21	17.82	1.08	C19H34O2	294	Linoleic acid, methyl ester	Fatty acid ester
22	17.87	3.49	C18H30O2	278	Linolenic acid	Fatty acid
23	17.95	1.38	C20H40O	296	Phytol	diterpene
24	17.99	0.65	C19H36O2	296	14-Octadecenoic acid, methyl ester	Fatty acid
25	18.14	6.66	C19H34O2	294	8,11-Octadecadienoic acid methyl ester	Fatty acid
26	18.20	7.73	C18H30O2	278	Gamolenic acid	Fatty acid
27	18.27	1.35	C21H38O4	354	1-monoLinolein	Fatty acid
28	18.45	0.97	C39H76O3	593	Oleic acid, 3-(octadecyloxy)propyl ester	Fatty acid
29	19.09	0.98	C23H48	324	Tricosane	alkane
30	19.74	0.34	C25H44N2O5S	484	2-Myristynoyl pantetheine	vitamin
31	20.42	0.56	C16H34S	258	Tert-Hexadecanethiol	thiol
		100.3			Total %	



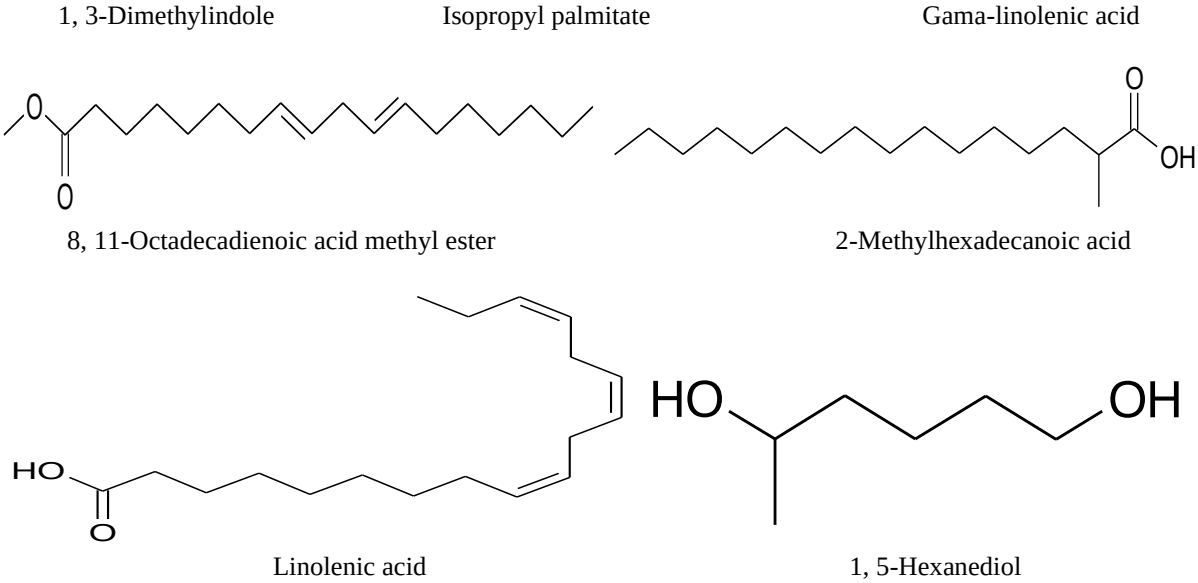


Fig. 2: The structure of main constituents of methanol extract of *P. emblica*

6. Conflicts of interest

There are no conflicts of interest between authors.

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