

# Anti-bacterial, Antioxidant activity and Phytochemical study of *Diospyros wallichii*—an Interesting Malaysia's endemic species of Ebenaceae

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**Abstract:** The traditional use of herbal medicine has increased significantly in these recent years. Asian countries are large producers of natural products and many of the products that we use today have their roots in the herbal traditional medicine. People are looking to save money, be environmentally conscious, use healthier and safer products. It goes without saying that the chemical antibacterial products are creating more resistant bacteria day by day, on the other hand herbal antioxidant agents have been proven to show less side effects. Therefore, it is logically and economically advised that countries, with enrich natural sources, start to formulate more natural products. The first studies on the therapeutic properties of the fruits and leaves extracts obtained from *Diospyros wallichii* King& Gamble in Malaysia are reported. In the present research the hexane, chloroform and ethanol extracts of *Diospyros wallichii* (fruits and leaves) are screened for antimicrobial and antioxidant activities. The study provides data to highlight the medicinal values of *D. wallichii*. Antibacterial activity was determined against *Bacillus cereus* ATCC10876, *Staphylococcus aureus* ATCC11632, *Pseudomonas aeruginosa* ATCC10145 and *Escherichia coli* ATCC10536 using the disk diffusion method. Results show that the hexane extract of fruits was active against both Gram negative and Gram positive bacteria. The chloroform extract of fruits had the highest free radical scavenging activity. The results obtained suggest that *Diospyros wallichii* King& Gamble. could be exploited in the development of therapeutic medicines and health care products.

**Keywords:** *Diospyros wallichii*, Ebenaceae, Anti-bacterial, Anti-oxidant, Natural products.

## INTRODUCTION :

Studying the medicinal plants as a source of biologically and pharmacologically active compounds has increased dramatically in past few years. Many plants and their components have been used as traditional medicines in many different parts of the world to cure various diseases [1-3]. Many of these herbs with high medicinal activities abound in the

tropical regions and rain forests [4]. These herbs elaborate a broad array of natural products, which give them the ability to manifest strong anti-oxidant and anti-bacterial activity [5-7]. The genus *Diospyros* from the family of Ebenaceae is found in the tropics and subtropics areas. Many species of *Diospyros* are medicinal and known to accumulate substantial amounts of naphthoquinones [8,9]. To date, there have

been no investigations done on the fruits and leaves extracts of *Diospyros wallichii* King & Gamble (Ebenaceae) in Malaysia. Due to the lack of new economical synthesized antibiotics and antioxidants, it is vital to look for the new substances from alternative medicines [10-12]. In continuation of our earlier studies on the pharmacological properties of Malaysian plants [13,14], this study was undertaken to screen the phytochemical composition, antibacterial and radical scavenging activities of different extracts of fruits and leaves of *Diospyros wallichii* King & Gamble.

#### **MATERIALS AND METHODS:**

**Chemicals:** Ascorbic acid and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Chemical Company. All solvents used in this study were obtained from R & M Marketing, Essex, UK. Muller Hinton agar culture medium (MHA) and Trypticase Soy broth (TSB) were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai, India. All other chemicals used in this study were of reagent grade.

**Plant material:** *Diospyros wallichii* King & Gamble. was collected from Ipoh (Perak, Malaysia) in December 2010 and was authenticated in the Forest Research Institute Malaysia (FRIM) Herbarium. The plant sample is also deposited as plant UNMC-63 at our own collection (School of pharmacy, Faculty of Science, Nottingham University) for future references.

**Preparation of extracts:** Freshly collected plant material (leaves and fruits) was dried in shade, and then coarsely powdered. The dried fruits (1.1 kg) and leaves (2.5 kg) were separately and consecutively macerated with solvents of increasing polarity (hexane, chloroform and ethanol) over a period of two weeks. The solvents were evaporated off under reduced pressure, and finally 6 extracts were stored at -20 °C until tested. The yields of extracts are presented in Table 1.

**Phytochemical screening:** All the six solvent extracts (hexane, chloroform and ethanol) of leaves and fruits were evaluated for the presence of different phytochemicals (Table 1) using procedures of Mojab et al. (2003).

**Bacterial strains:** The microorganisms used in this study consisted of four strains. *Bacillus cereus* (ATCC 10876; Gram-positive), *Staphylococcus aureus* (ATCC 11632; Gram-positive), *Pseudomonas aeruginosa* (ATCC 10145; Gram-negative) and *Escherichia coli* (ATCC 10536; Gram-negative). All bacterial strains were obtained from the School of biosciences, Faculty of Science, Nottingham University Malaysia Campus. The bacteria were grown and maintained on Trypticase Soy agars (TSA) at 37 °C overnight.

**Antibacterial Assay:** The anti-bacterial bioassay was based on disc diffusion method expressed by the National Committee for Clinical Laboratory Standards (NCCLS) (2002) using bacterial cell trypticase toy broth (TSB) suspension in which the concentration was equilibrated to a 0.5 McFarland standard. The bacteria's suspension of each strain was spread on a Mueller–Hinton agar plate. Sterile paper discs (6 mm diameter) were impregnated with 10 µL of each extract dissolved in dimethylsulfoxide (DMSO) used for the concentration at 2mg/disc. The discs were allowed to dry completely and then placed on the inoculated agar. Discs with the solvent used for dissolution were used as negative control and 10 µg streptomycin discs were used as positive controls. The plates were incubated at 37 °C for 24 hours. Clear inhibition zones around the discs indicated the presence of antibacterial activity. After the incubation time, zone of inhibition was measured precisely. The assay was carried out in triplicates and was repeated for several times. The strength of activity was classified as very strong (++++), for inhibition zone diameters (i.d.) > 25 mm, strong (+++) for diameters ranging from 24 to 15 mm, moderate (++) for diameters 14-11 mm and weak (+) for diameters 10-8 mm (Table 2).

**Antioxidant capacity:** The method of DPPH radical scavenging assay, which was based on using the 96 well plates, was performed following the Habtemariam et al. 2007 procedure. The test sample dilutions were prepared with methanol and DMSO as required solvents. After 20 min of incubation in the darkness at room temperature, absorbance at 550 nm was measured and percentage DPPH scavenging activity was calculated [15].

**Statistical analysis:** All experiments were carried out at least in triplicate. Statistical analysis was performed using MS-Excel software.

**Table1. Phytochemical constituents of *Diospyros wallichii* King & Gamble and yield of extraction.**

Phytochemical	Flavonoids	Tannin	Steroid	Saponin	Poly phenol compounds	Yield of Extraction %
Leaf Hexane	-	-	-	-	+	3.1
Leaf Chloroform	-	-	-	-	+	2.3
Leaf Ethanol	-	-	-	+	++	1.2
Fruit Hexane	-	-	+	-	++++	0.1
Fruit Chloroform	-	-	+	-	+++	1.7
Fruit Ethanol	-	-	-	+	++	1.1

**Table 2. Growth inhibitory responses of various fractions obtained from the extract of *Diospyros wallichii* King & Gamble.**

Fraction <sup>a</sup>	Bacterial species <sup>b</sup>			
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
Hexane leaf fraction	-	-	-	-
Chloroform leaf fraction	-	+	-	-
Ethanol leaf fraction	++	++	-	-
Hexane fruit fraction	+++	++++	-	++
Chloroform fruit fraction	++	+++	-	-
Ethanol fruit fraction	+	+	-	-
Streptomycin (10µg/disc)	++	++	++	++
Negative Standard	Complete growth			

<sup>a</sup> Exposed to 2 mg/disc.<sup>b</sup> Cultured on molten hinton agar at 37 °C for 1 day.<sup>c</sup> Zone of inhibition diameter >25 mm,++++; 24–15 mm, +++; 14–11 mm, ++; 10-8 mm, +; <8 mm,-.

## RESULTS:

The preliminary phytochemical study of the extracts of *Diospyros wallichii* King & Gamble revealed the highly presence of poly phenols in all six extracts tested. The results of phytochemical study are shown in Table1.

The growth-inhibiting effects of the extracts obtained from the leaves and fruits were assayed using the paper disc agar diffusion method against 4 strains of bacteria. During the screening, the fruit hexane extract exhibited a very strong inhibiting activity (++++) at a concentration of 2.0 mg/disc against *Staphylococcus aureus* and also it manifested strong inhibiting activity (+++) against *Bacillus cereus* (Table 2).

The results indicate that the fruit hexane and the fruit chloroform extracts of *Diospyros wallichii* King & Gamble contain compounds with high bactericidal activities against *Staphylococcus aureus* and *Bacillus cereus*. Moreover the inhibition zone obtained against *Escherichia coli* strain was amazingly considerable. The activities observed in the fruit extracts of *D. wallichii* strongly support the future industrial use of this plant as a bio-source for producing novel anti-bacterial commodities.

DPPH radical scavenging assay method has been used to measure the antioxidant activities of the obtained extracts. After compromising the scavenging effects of all the tested extracts, the results illustrated that the fruit chloroform extract has the highest activity among all other tested samples (Table 3).

**Table 3. The percentage of Radical-scavenging activity on DPPH by extracts of *Diospyros wallichii***

Concentration (µg/ml)	Fruit Hexane Extract	Fruit Chloroform Extract	Ascorbic Acid
100	78.15±3.66	95.50±4.17	93.02±2.85
50	51.72±3.27	86.25±2.56	89.38±5.71
25	29.16±4.05	66.57±2.04	75.76±4.60
12.5	24.54±3.06	50.39±1.93	44.15±2.70
6.25	14.20±3.03	33.03±1.97	23.43±3.23
3.125	9.11±3.00	30.10±2.03	21.91±4.08

a) Mean values ± standard deviations.

The data show that the fruit hexane and chloroform extracts are free radical inhibitors and can act as primary antioxidants. These results can confirm earlier studies done on other *Diospyros* species [16-19]. The results show that the radical scavenging activity of the fruit chloroform extract of *Diospyros wallichii* is more comparable to the ascorbic acid. It was also observed that the extract of the fruit chloroform had even higher radical scavenging activity than the hexane extract. At a concentration of 100 µg/ml, the scavenging activity of the fruit chloroform extract reached to 95.50%, while at the same concentration, fruit hexane extract was only 78.15%.

#### **DISCUSSION:**

This study assessed the anti-bacterial and the anti-oxidant activity of the six fractions obtained from the leaves and fruits of *Diospyros wallichii* King& Gamble. There is a compelling body of evidence that indicates that naphthoquinones abound in the genus of *Diospyros* [20-24] so it can be concluded that these agents are possibly responsible for the observed anti bacterial activities. Another interesting fact is that *Diospyros wallichii* King& Gamble exhibited antibacterial activities which were comparable to streptomycin (0.1 mg/ml). Most studied natural products show growth inhibiting properties against Gram-positive bacteria. The fruit hexane extract of *Diospyros wallichii* King& Gamble exhibited strong inhibiting activities against the Gram-negative bacteria *Escherichia coli*. Although some may still claim that the concentration of 2 mg/disc is much, the experiment showed us that this plant has a broad spectrum antibacterial activity in in-vitro conditions.

Polyphenols are the major plant compounds with the antioxidant activity. This activity is believed to be mainly due to their redox properties [25]. Results of the present study reveal that the levels of these phenolic compounds were considerable in the hexane and chloroform fruit extracts of *D. wallichii* King& Gamble (Table 1) which elucidate moderate and strong anti-oxidant activity, respectively.

#### **CONCLUSION:**

Nowadays, medicinal plants play a crucial role in facing with many diseases. Plants are not only studied as traditional medicines but also they have independently started a new line of commercial natural products. In addition, the Malaysian rainforest plants, which have not been completely investigated, can be identified as one of the main natural sources for pharmaceutical industries. Therefore, our study demonstrates that the Malaysian *Diospyros wallichii* King& Gamble holds tremendous potentials as source of antibiotics, antiseptics and food preservatives [26,27]. To achieve the mentioned goals our team is currently conducting further phytochemical investigations in order to standardize new potent fractions and agents.

#### **ACKNOWLEDGEMENTS:**

We are grateful to FRIM for plant identification and to Mr.Hasan for cooperation to collect plant materials in Perak. The financial support of Azm-E-Rah and Jalinus Co-operations in performing the computational studies is gratefully acknowledged.

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