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ANTIBACTERIAL AND FREE RADICAL SCAVENGING ACTIVITY OF *DURANTA PLUMIERI,* LINN.

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ABSTRACT

The antibacterial properties of methanol leaf extract of *Duranta plumieri* was determined against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis and Bacillus subtilis* as test organisms using the agar disc diffusion method. The minimum inhibitory concentrations were determined using the microbroth dilution method. The methanol extract was subjected to preliminary phytochemical analysis. Free radical scavenging activity of the methanol extract at different concentrations was determined with 2, 2-diphenyl-1picrylhydrazyl (DPPH). *E. coli, Staph. aureus, Kl. pneumoniae, B. subtilis* and *Pr. mirabilis* were susceptible to *D. plumieri* extract with minimum inhibition concentrations of 48 mg/ml, 192 mg/ml, 192 mg/ml, 12 mg/ml and 96 mg/ml respectively. The extract had no activity against *Ps. aeruginosa.* Alkaloids, glycosides, saponins, steroids and polyphenols (tannins and flavonoids) were detected as phytoconstituents of the methanol extract.

INTRODUCTION: Today more people than ever before are questioning the cycle of diagnosis and prescription that conventional physicians practice, and are taking increasing responsibility for maintaining their own health and wellbeing. The medicine practiced by our forefathers has been the precursor of modern pharmaceutical medicine. This includes herbal medicine and phytotherapy, which is prevalent in Chinese, Ayurvedic (Indian), and Greek medicine [1]. Many synthetic medicines such as quinine and aspirin, commonly used in modern medicine, had their roots in traditional medicine. Furthermore, morphine, is an active element of the opium plant atropine, comes from belladonna leaves and the heart drug digitoxin from leaves of the foxglove plant [2].

Nowadays, the prevention of many diseases has been associated with the ingestion of different plants rich in antioxidants [3]. It was found out that intake of such compounds is associated with a lower risk of mortality from different kinds of diseases [4]. The protective effects of plants are due to the presence of several components such as proteins, vitamins carotenoids, flavonoids (anthocyanins) and other phenolic compounds [5, 6].

Duranta plumieri (Linn) belongs to the family Verbenaceae, a native of tropical America and is commonly known as golden dew drop. It is widely cultivated in Ghana as an ornamental plant as a result of its ease of propagation and its beautiful colouration. *D. plumieri* is an important plant which possesses some important medicinal properties. The leaves contain saponins and macerated fruits are lethal to mosquito larvae and it also possesses antifungal activity when applied topically [7]. In Ghana some herbalist use its' leaves to manage wounds and skin afflictions. Aqueous extracts of seeds of *D. plumieri* is rich in polyphenol oxidase activity [8]. In this study extracts of this plant were screened for antibacterial and antioxidant activities.

MATERIALS AND METHODS:

Plant material: Leaves of *Duranta plumieri* (Linn), Verbenaceae, were obtained from The Kwame Nkrumah University of Science and Technology campus and authenticated at the Department of Pharmacognosy, where a herbarium specimen (FP/094/09) has been deposited. The plant parts were washed and sun dried for 7 days and then milled into coarse powder using a laboratory Mill Machine (Type 8, Christy & Norris, UK).

Preparation of methanol extract: 125g of the powdered leaves were Soxhlet extracted using 70% methanol and concentrated under reduced pressure using a Buchi Rotavapor R-114. The concentrate was evaporated to dryness at 40^{o}C in a hot air oven. The weight of extract obtained was 32.18g giving a yield of 25.74% (w/w). The extract was stored in an airtight container at 4°C.

Microorganisms used for the tests: The antibacterial activity of the methanol extract was determined by individually testing on Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis,* NCTC 10073), Gram-negative bacteria (*Klebsiella pneumoniae* ATCC 31488, *Proteus mirabilis* ATCC 12453*, Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922).

Preliminary phytochemical tests: The methanolic extract was phytochemically screened for tannins, glycosides, saponins, alkaloids, flavonoids and steroids using the procedures outlined by Wall *et al*, (1952) [9] and Harbone (1973) [10].

Antimicrobial activity determination: The antimicrobial activity was determined using the agar disc diffusion method. Overnight cultures were grown at 37°C in Muller-Hinton Broth (MHB) (Sigma-Aldrich, St Louis, MO, USA) and diluted to contain 10^5 cfu/ml. Petri dishes containing 20 ml of Muller-Hinton Agar (MHA) (Sigma-Aldrich, St Louis,

MO, USA), were used for the disc diffusion assay. 200 µl of the bacterial culture was spread over the surface of the plate and allowed to dry for 10 minutes. Filter paper discs (6 mm in diameter) were soaked with 30µl of various concentrations (400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml) of extract in water and placed on the inoculated agar. Discs containing tetracycline (10 µg/disc) were placed as control. These were incubated at 37^{o}C for 24 hours. The antibacterial activity against each test organism was quantified by determining mean zone of inhibition.

Determination of minimum inhibitory concentration: The minimum inhibitory concentrations (MIC) of the extract against the various organisms were determined using the microwell dilution method [11]. The 96-microwell sterile plates were prepared by dispensing 250µl of inoculated broth and 50µl of plant extract constituted in broth or 50µl broth (MHB) in the case of negative control in each well. Tetracycline (Sigma-Aldrich, USA) was included as positive control. The plates were incubated at 37^{o}C for 24 hours. Presence of bacterial growth was determined by the addition of 50µl of p-iodonitrotetrazolium violet (0.2 mg/ml, Sigma).

Determination of Antioxidant activity: Determination of the antioxidant activity was done using the 2, 2 diphenyl-1-picryl hydrazyl (DPPH) assay method. A 0.004% w/v of DPPH was prepared in methanol and stored away from light. Different concentrations of the plant extract in methanol (1.875 µg.ml, 3.75 µg/ml, 7.5 µg/ml, 15 µg/ml and 30 µg/ml) were also prepared. 50µl each of the five concentrations of the extract was added to 5ml of 0.004% DPPH. 0.004% DPPH in methanol was used as blank. The tubes were incubated at room temperature for 30 minutes and their absorbance was read at 517nm on a Thermo Spectronic UV spectrophotometer. The scavenging activity was observed by bleaching of the colour of DPPH solution from violet to light yellow. The procedure was repeated for similar concentrations of N-propyl gallate solutions as reference standard.

The DPPH radical scavenging activity was calculated as

$$\% \text{ Inhibition} = [100(Ac-As)]/Ac,$$

Where *Ac* is the absorbance of the blank and *As* is the absorbance of the sample.

The IC_{50} was obtained through extrapolation from analysis using Prism software version 5.

RESULTS: The phytochemical analysis of the methanol extracts showed the presence of tannins, glycosides, flavonoids, saponins, steroids and alkaloids. The methanol extract of *D. plumieri* had antimicrobial activity against all the test organisms except *Ps. aeruginosa* though the activity was far less than the standard (tetracycline) used (**Table 1**); the MICs against the various organisms are as shown in **Table 2**.

TABLE 1: ZONES OF INHIBITION OF EXTRACT AGAINST VARIOUS ORGANISMS

Organism	Zone of inhibition (Mean ±SEM, mm)				
	Extract 50 mg/ml	Extract 100 mg/ml	Extract 200 mg/ml	Extract 400 mg/ml	Tetracycline 10 µg/ml
E. coli	9.0±0.2	10±0.9	11±0.3	12±0.13	19±0.14
Staph. aureus	5.0±0.1	7.0±0.14	8.0±0.2	9.0±0.8	14±0.12
Ps. Aeruginosa	--	--	--	--	9.0±0.22
Kl. Pneumonia	4.0±0.3	7.0±0.7	8.0±0.3	10±0.05	16±0.13
B. subtilis	8.0±0.6	11±0.4	12±0.1	14±0.17	18±0.04
Pr. Mirabilis	8.0±0.21	9.0±0.8	11±0.4	13±0.21	22±0.01

Key: -- no zone of growth inhibition observed

TABLE 2: MIC OF METHANOL EXTRACT AGAINST VARIOUS ORGANISMS

Organism	MIC (mg/ml)
E. coli	48
Staph aureus	192
Ps. aeruginosa	0
Kl. pneumonia	192
B. subtilis	12
Pr. mirabilis	96

The methanol extract of this plant exhibited scavenging activity with IC_{50} = 22.5 µg/ ml while that of n-propyl gallate was <1.875 µg/ml. **Table 3** shows the scavenging activity of the methanol extract at different concentrations. The scavenging activity was seen to increase gradually with increase in concentration of extract; however, the scavenging activity was low in comparison with the standard, n-propyl gallate.

TABLE 3: FREE RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF *D PLUMIERI*

Concentration (µg/ml)	% Scavenging Activity	
	N-propyl gallate	*D. plumieri*
30	80.0	58.18
15	73.28	37.22
7.5	60.56	14.19
3.75	58.43	10.55
1.875	53.88	0.81

DISCUSSION: The study found the presence of alkaloids, glycosides, saponins and polyphenols such as tannins and flavonoids in the methanol extract of *D. plumieri*. The extract was found to have both antibacterial and antioxidant activity. It exhibited antibacterial activity against all the organisms used for the study except *Ps. aeruginosa*. The antimicrobial activity is demonstrated by the establishment of zones of growth inhibition around the filter paper discs. For those organisms that showed sensitivity to the extract *B. subtilis* was the most sensitive (MIC 12 mg/ml) while *Staph. aureus* and *Kl. pneumoniae* were the most resistant (MIC 192 mg/ml). The range of MIC values obtained indicates that the antimicrobial metabolites may be present in very small quantities or may not be very potent against the test organisms used.

The phytochemicals found may exert antimicrobial activity through different mechanisms, for example tannins exhibit antimicrobial action through inactivation of microbial adhesions, enzymes and cell envelope transport proteins, and forming complexes with polysaccharides [12]. This action causes destruction of the cell walls of both gram positive bacteria and the lipopolysaccharides layers of gram negative bacteria leading to the death of these microorganisms [13].

Tannins are known to have antimicrobial and astringent activity and play a very important biochemical role in wound healing [14]. Such roles include binding to proteins of exposed tissues, thus precipitating the proteins, and forming antiseptic protective coat which enables the regeneration of new tissues to take place. Thus the antibacterial activity of the extract will have a positive effect on wound healing.

Some of the constituents, especially the polyphenols, are responsible for the antioxidant activity established. The effect of antioxidants molecules on DPPH is due to their hydrogen donating ability [15]. The study shows that the extract has antiradical action and serves as free radical scavenger though its activity as an antioxidant is weak (IC_{50} = 22.5 µg/ml) compared with the standard (n-propyl gallate, IC_{50}<1.875 µg/ml). Thus, the presence of the above phytochemicals corroborates the scavenging activity exhibited by the methanol extract of *D plumieri*, and supports any possible use in the treatment of wounds and burns. It can be seen that for equal concentrations of *D. plumieri* extract and n-propyl gallate, the amount of DPPH scavenged or absorbed by the n-propyl gallate is far greater than

that scavenged by the *D plumieri* extract (table 3). The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The presence of antioxidant phytochemicals in the extracts (example, tannins and flavonoids) is possibly responsible for the free radical scavenging activities exhibited by the plant.

It has been established that tannins and flavonoids have strong free radical scavenging and anti-inflammatory activities [16]. Flavonoids possess antioxidative and radical scavenging properties and regulate cellular activities of the inflammation-related cells which include mast cells, macrophages, lymphocytes, and neutrophils. For instance, some flavonoids inhibit histamine release from mast cells and others inhibit *t*-cell proliferation [17].

Antioxidants are also included in formulations to prevent deterioration from oxidation. In this regard antioxidants are grouped into three categories. The first, true antioxidants or anti-oxygens probably inhibit oxidation by reacting with free radicals. The second which are reducing agents, have a lower redox potential than the drug or adjuvants which they are intended to protect and are therefore more readily oxidised. The third are antioxidant synergists which usually have little antioxidant effect themselves but probably enhance the action of true antioxidants by reacting with heavy metal ions which catalyse oxidation [18]. Antioxidants from plant sources such as found in this study may prove very useful in pharmaceutical formulations.

CONCLUSION: The methanol extracts of *D. plumieri* was found to contain tannins, glycosides, flavonoids saponins, steroids and alkaloids. It showed antibacterial activity against *E. coli, Staph. aureus, B. subtilis, Pr. mirabilis and Kl. pneumoniae.* It had no activity against *Ps aeruginosa.* The methanol extract had antioxidant activity with an IC_{50} value of 22.5 µg/ml while the reference standard (n-propyl gallate) had an IC_{50} value of <1.875 µg/ml. The antioxidant activity of the methanol extract of *D plumieri* is low compared to the reference standard.

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