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EFFECT OF CONCENTRATION ON THE ANTIMICROBIAL ACTIVITY OF PHYTOCHEMICALS FROM *GUIERA SENEGALENSIS* LEAVES

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Abstract

Fresh leaves of Guiera senegalensis were washed, air dried and pulverized. Aqueous and methanolic extracts of the leaves were obtained by soaking in distilled water and soxhlet extraction using methanol, respectively. The extracts were subjected to phytochemicals screening according to the standard methods. Bacterial and fungal species were used as test organisms. Agar well diffusion method was used for the determination of the antimicrobial activity of the pyotochemicals. The concentrations (mg/cm^3) of the methanolic extract used were 100, 150, 200, 250, and 300, and the plates were labeled A, B, C, D and E, respectively. The standard drugs used as control was ciprofloxacin (cf). The work showed that all the phytochemicals screened were present in the leaves; the activities of the phytochemicals on the bacterial species were positive at all the concentrations used and negative on the fungal species at concentrations below 300g/cm^3 with least inhibitions at 300mg/cm^3 and; the antibacterial activities of the phytochemicais were proportional to the concentration of the phytochemicals.

Keywords: *Guiera senegalensis leaves; phytochemicals; concentration; test organisms; anti microbial activity.*

Introduction

Over centuries, medicinal plants have been used all over the world for treatment and prevention of various ailments, particularly in developing countries (Nigeria inclusive), where infectious diseases are edemic and modern health facilities are grossly inadequate. In the whole world, traditional medicine is of great value and, more than 80% of the people refer to traditional healers concerning health issues (Abdulsalam *et al*, 2004). In Nigeria as in other African countries, roots leaves, fruits, seeds and barks of different plants are used for various medicinal purposes (Jinju, 1990).

Alternative forms of health care coupled with the resistance of some disease causing organisms to the available antibiotics have led many researchers to investigate the antimicrobial activity of medicinal plants (Maoz and Neema, 1998). The medicinal value of plants lies on some bioactive phytochemicals that produce a definite physiological action on human body (Akinnoladun *et al,* 2007). Phytochemicals are natural bioactive compounds presents in plants, which are responsible for colour and other organoleptic properties (Olayemi *et al*, 2014). Phytochemicals work together with the nutrients and fibre to act as defense system against diseases (Olayemi *et al*, 2014). Presence of secondary metabolites (phytochemicals) in plants has been reported to have antimicrobial activity (Hostettman *et al*, 1995; Isaac and Chinwe, 2001).

Guiera senegalensis known as "*Sabara*" in Hausa is a shrub with whitish dusty –looking leaves, or which belongs to a family combretaceae. It is abundantly in dried infertile regions with little rainfall. The leaves, the stems and the roots of *Guiera senegalensis* are used in various ways for medicinal purpose (Jinju, 1990). This work reports studies on phytochemicals of *guiera senegalensis* leaves as well, as the effect of concentration of the phytochemicals on the antibacterial and fungal activities.

Materials and Methods

Collection, Identification and Treatment of the Experiment Plant.

The plant *Guiera senegalensis* from which the leaves were obtained was collected from Dunawa grazing land located in Dawakin Tofa Local Government Area, Kano State-Nigeria. The leaves were fresh and mature at the time of collection. The plant was carefully examined and identified by a taxonomist in Botany Department of the University of Jos-Nigeria. The leaves were first washed under running tap water sourced from a mechanized borehole and air-dried (indoor) for 10 days, after which they were pulverized into powder using wooden pestle and motar and subsequently sieved to a mesh size of less than 250µm using mesh of well defined particles sizes according to the method described by Isaac and Chinwe (2001).

Collection of Bacterial Culture

The pure cultures of the four (4) bacterial species used for the antibacterial investigation were collected from microbiology laboratory of the University of Jos Teaching Hospital (JUTH), Plateau State, Nigeria. The bacterial species collected were Escherichia coli (Ec), Klebsiella pneumonia (Kp), Pseudomonos aeroginosa (Pa) and Staphylococcus aureus (Sa). The bacterial species were identified according to the method adopted by Abduisalam *et al* (2014).

Collection of Fungal Species

The four (4) fungal species: Aspergillus niger (An), Cuvularia lunata (Cl), Fusarium oxysporum (Fo), and Penicillum citrimun (Pc) were collected and isolated in pure form from a mixed culture harnessed from air on a malt extract in agar. The fungal species were identified by fixing them on microscope and then compared with the known structures in the literature (Ekwenchi *et al,* 1990).

Extraction of Aqueous Extract of the Leaves

The aqueous extract of the leaves was extracted by soaking of 50.00 g of the powdered leaves in 1dm^3 of distilled water and kept at room temperature for 24 hours. The supernatant liquid (extract) formed was filtered off using Whatman filter paper (18.50 cm diameter).

Extraction of Methanolic Extract of the Leaves.

The methanolic extract of the leaves was extracted by soxhlet extraction, where 100.00 g of the sample (powdered) leaves of *Guiera senegalensis* were packed in muslin cloth and placed in a thimble. The extraction was carried out at 85^{o}C for 8 hours using methanol as extraction solvent. The extract was recovered from the extraction solvent by evaporation using rotary evaporator and subsequently concentrated to a constant weight by heating at 37^{o}C on hot plate.

Phytochemicals Screening

The phytochemicals were screened according to standard methods as described below:

For the screening of alkaloids, 0.500 g of the concentrated methanolic extract was treated with iodine in potassium iodide (Wagner's reagent). Formation of brownish-red precipitate indicates the presence of alkaloids (Edeoga *et al*, 2005).

The flavonoids content of the leaves was screened using the aqueous extract, where 5.00 cm^3 of dilute ammonia solution were added to a small portion of the aqueous extract followed by the addition of concentrated H_2SO_4. Formation of yellow coloration indicates the presence of flavonoids (Harborne, 1973).

A quantity measure of 2.00 g of the concentrated methanolic extract were treated with 3-4 drops of $FeCl_3$ solution. Formation of blue –black colour indicates the presence of phenols (Harborne, 1973).

For the screening of saponines, 0.50 g of the concentrated methanolic extract was vigorously shaken with 2.00 cm^3 of distilled water and then left to stand for 10 minutes. Formation of a thick persistent growth (foam) indicates the presence of saponines (Sofowora, 1993).

The steroids content of the leaves was analysed (screened) by the treatment of a chloroform solution of the powdered leaves with acetic anhydride and a few drops of a concentrated H_2SO_4 was added down the side of the test-tube. A blue green ring indicates the presence of steroids (Ibe, 2014).

The tannines content of the leaves was screened by mixing 2.00 g of the concentrated methanolic extract with distilled water and heated on a waterbath. The mixture was filtered and $FeCl_3$ solution was added to the filterate. Formation of green solution indicates the presence of tannines in the sample (Sofowora, 1993).

A small quantity of the sample was dissolved in ethanol. 1.00 cm^3 of acetic anhydride was added to the mixture followed by the addition of concentrated H_2SO_4. A colour changes from pint to violet indicate the presence of terpenoids in the leaves (Ibe, 2014).

Determination of Antimicrobial Activity of the Methanolic Extract of the leaves at Concentrations

In order to determine the antimicrobial activity of the methanolic extract of the leaves, agar well diffusion method was used.

Antibacterial Activity

For the study of the antibacterial activity of the leaves extract, plates were prepared by pouring sterile Muller Hilton agar into sterile petri-dishes that were previously autoclaved at 115^oC. Sterilized cotton swabs were dipped into the bacterial culture in a nutrient agar broth and subsequently swabbed on the plates. Wells of equal sizes were bored with proper gaps in the agar medium on the plates using cork borer. Concentrations (100mg cm^3, 150mg cm^3, 200mg cm^3, 250mg cm^3 and 300mg cm^3) of the concentrated methanolic extract were prepared using distilled water and added into the plates, which were labeled A, B, C, D and E, respectively. The plates were incubated at 37^oC for 24 hours. The standard drug used as control was Ciprofloxacin (C_f), which was prepared by dissolving the drug in distilled water at concentration of 1mg/cm^3 and, distilled water was used as blank. After the incubation, inhibition zones were measured in millimeter (mm). The determination was carried out in triplicate and the average of inhibition zone on each plate was evaluated and, the inhibition zone of the blank (distilled water) was subtracted from the inhibition zone on each plate.

Antifungal Activity

For the determination of the antifungal activity of the leaves extract, the same procedure and conditions as in the case of antibacterial were employed but in this case, different fungal species were used as test organisms, instead of bacteria.

Results and Discussion

The results of all the analyses carried out are shown in Tables 1-3 below. Table 1 shows the phytochemical contents of the leaves. Table 2 gives the antibacterial activity of the methanolic extract of the leaves at different concentrations. Table 3 shows the antifungal activity of the methanolic extract of the leaves at different concentration.

Table 1. Phytochemicals Contents of the Leaves of *Guiera senegalensis*

S/N	Phytochemicals	Inference
1.	Alkaloids	+
2.	Flavonoids	+
3.	Phenols	+
4.	Saponines	+
5.	Steroids	+
6.	Tannines	+
7.	Terpenoids	+

Key: + = Present

Table 2: Antibacterial Activity (mm) of the Methanolic Extract of the Leaves at Different Concentrations.

S/N	bacterial Species	concentrations of methanolic extract (mg/cm^3)					concentration of C_f
		100	150	200	250	300	(1mg cm^3)
1.	Fc	18.00	21.00	25.00	26.00	26.00	0.50
2.	Kp	10.00	14.00	18.00	21.00	22.00	2.00
3.	Pa	19.00	22.00	25.00	27.00	28.00	2.00
4.	Sa	20.00	24.00	27.00	27.00	31.00	2.50

Table 3: Antifungal Activity (mm) of the Methanolic Extract of the Leaves at Different Concentrations.

S/N	fungal species	concentration of methanolic extract (mg/cm^3)					concentration of C_f
		100	150	200	250	300	(1mg cm^3)
1.	An	NA	NA	NA	NA	2.00	NA
2.	Cl	NA	NA	NA	NA	2.50	NA
3.	Fo	NA	NA	NA	NA	2.50	NA
4.	Pc	NA	NA	NA	NA	2.50	NA

Key: NA= No activity.

Discussion

The result of the phytochemicals screening shown in Table 1 revealed the presence of all phytochemicals screened in the leaves. These phytochemicals (secondary metabolites) have been found to be responsible for the various therapeutic activities of medicinal plants (Abdussalam *et al*, 2014). The presence of these secondary metabolites in the leaves could serve as index for the various medicinal uses of the leaves of *Guiera senegalensis* in different ways for the treatment of different sickness by most rural populace. This is in line with the statement of Olayemi *et al,* (2014), which says the antimicrobial activities and biological importance of plants lie on the phytochemical contents of the plants.

The result of antibacterial activity of the methanolic extract of *Guiera senegalensis* at different concentrations is shown in Table 2. From the results, it could be seen that there was activity on each of the test organisms (bacterial species) tested. This may be attributed to the presence of some bioactive phytochemicals in the plants, which work together with the nutrients and fibre to acts as defense system against diseases (Olayemi *et al*, 2014). In addition, the presence of some bioactive phytochemicals provides a definite physiological action on human body

(Akinnoladun *et al*, 2007). This could be a reason for using plants (*Guiera senegalensis* inclusive) in the treatment of various sicknesses especially by the rural populace. The inhibition zones observed on the plates were found to be concentration dependent in which high inhibition were recorded at high concentration. This could be associated with the presence of naturally existing metallic elements such as K, Na, Mg, Fe, Cr, Pb, Cu, Zn and so on. These elements have reported to have some antimicrobial effects due to the role they play in chemotherapy, protein synthesis, action of many emzymes and many other essential functions in human and animal health (Olayemi *et al*, 2014). This could also be a reason for using the plant (*Guiera senegalensis*) as medicinal plant by the majority of traditional healers.

Table 3 shows the antifungal activity of the leaves at different concentration of the methanolic extract. The result showed that there was no activity on each of the test organism tested at extract concentration below 300 mg/cm^3, so also with the standard drug (C_f) used. The little inhibition observed at 300 mg/cm^3 extract concentration may be connected to the high concentration of the secondary metabolites. It could also be connected to the presence of other secondary metabolites which were not investigated in this work. In addition, the presence of some naturally existing metallic elements in the leaves at high concentration could also be a factor responsible for the little inhibition observed at relatively high concentration (300mg/cm^3) due to their antimicrobial effect as reported by Olayemi, *et al* (2014).

Conclusion

Based on the findings of the present work and considerable biological importance of phytochemicals, the leaves of *Guiera* senegalensis could be used (after thorough investigation and purification) as substrate for the manufacture of antibacterial drugs.

Recommendations

In order to explore more about the antimicrobial activity of *guiera senegalensis* and other medicinal plants, the following recommendations could greatly help:

1. Research should be conducted on the effect of temperature on antimicrobial activities of phytochemicals contents of medicinal plants.
2. Effect of catalyst and/or additives on the antimicrobial activity of phytochemicals from plants sources should be investigated.
3. Determination of the maximum allowable concentration (MAC), lethal dose of 50% (LD_{50}) of the disease causing microbes and inhibition concentration of 50% (IC_{50}) of the phytochemicals from some selected medicinal plants should be carried out.
4. Effect of light intensity on stability and potency of phytochemicals from plants sources should be investigated.
5. Research on preservation measures and stability enhancement of phytochemicals should be carried out.
6. Government at all levels and donor agencies should put hands together to support the production of drugs from available medicinal plants in our localities.
7. NAFDAC should strictly monitor and regulate the use of herbs for traditional medicines.

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