

Full Length Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF SOME POWDERED HERBAL PREPARATIONS MARKETED IN KADUNA METROPOLIS.

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ABSTRACT.

The aim of the study was to investigate the phytochemical components and the antibacterial activities of some powdered herbal medicinal preparations sourced from identified herbal shops and retail outlets in different parts of Kaduna metropolis. Extracts obtained from the herbal preparations were screened for the presence of secondary metabolites using established procedures. Also, antibacterial activities of the extracts were evaluated. Carbohydrates and tannins were identified in 105 (70%) and 101 (67.3%) of the samples respectively. Alkaloids were found in 97 (64.7%); saponins were detected in 91 (60.7%), while anthraquinones, flavonoids and cardiac glycosides were identified in 82 (54.7%), 80 (53.3%) and 60 (40%) of the herbal preparations respectively. All the methanolic extracts had inhibitory activities on the test bacterial isolates at various minimum inhibitory concentrations: 81 (54%) had inhibitory effects on *Staphylococcus aureus*, 74 (49.3%) on *Escherichia coli*, 74 (49.3%) on *Salmonella typhi* and 63 (42%) on *Shigella spp.* The uses of these products in herbal medicine are justified. However, further works are needed to identify the chemical nature of the active substances as well as their modes of actions on the bacterial cells and their roles in disease curing.

Keywords: Herbal, medicinal, phytochemical, antibacterial.

INTRODUCTION

Herbal preparations, also called phytochemicals, refer to the use of any plant's parts for medicinal purposes. Traditional medicine has been defined by Bhushan (2005) as diverse health practices, approaches, knowledge and beliefs incorporating plants, applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness. This is believed to have existed before the advent of orthodox medicine (Kossowan, 1991).

Herbal preparations are easily obtainable and can be used as extracts or as crude substances which can be further purified (Awosika, 1993). They are available in varieties, including fresh,

dried, tablets, capsules, or bottled in liquid forms. They may also be available either singly, or in mixtures formulated for specific conditions (Janatzen, 1994). Many researches have demonstrated the powerful roles that herbal medicines can play in preventing, relieving and treating serious life threatening diseases such as Acquired Immunodeficiency Syndrome (AIDS), as well as their utility in more practical applications such as immune-enhancement (Barrett, 2003). The leaves, flowers, stems, roots and other components derived from plants have been reported to be effective antibacterial agents (Nkere & Iroegbu, 2005).

In view of the increasingly difficult problems of microbial resistance to most antibiotics (Chen *et al.*, 2005), medicinal plants are now being considered as credible alternatives for the treatment of diverse infections. Many of them have been shown to have a variety of pharmacological effects, including anti-inflammatory (Chainani, 2003) and antimicrobial activities against wide range of pathogenic microorganisms (Musa *et al.*, 2000; Adeleye & Opiah, 2003; Khan *et al.*, 2003).

MATERIALS AND METHODS.

Study area and sampling: A total of one hundred and fifty (150) different herbal preparations were purchased randomly from identified herbal shops and retail outlets in different parts of Kaduna metropolis. Packaged herbal samples were obtained and taken to the laboratory, while those that were not packaged (such as those sold by local herbalists) were collected in sterile polythene bags (Pearce *et al.*, 2004). All samples collected from the sites were analysed in the laboratories of the Departments of Microbiology and Pharmacognosy, Ahmadu Bello University, Zaria, as well as the area laboratory of National Agency for Food and Drug Administration and Control (NAFDAC), Kaduna.

Phytochemical analyses: In a preliminary study, different solvents [water, methanol, ethanol, chloroform, ether and acetone] were used for the extraction of the herbal products (Igile *et al.*, 1994; Silva *et al.*, 2003). Maceration method was finally employed using n-Hexane and methanol as solvents. 250g of the powdered herbal sample were thoroughly mixed and defatted with n-Hexane. 150g of the dried powder was weighed separately into 1000ml conical flask and 500ml ethanol added and covered with aluminum foil. Homogenization of the mixture and saturation of the solvent was achieved by shaking mechanically for 24hrs using gyrator shaker at

100rpm. The mixture was filtered using sterile filter paper (whatman No.2). The filtrate was evaporated to dryness over a boiling water bath. The crude extract was then scrapped off from the bottom of the evaporating dish and stored in labeled bottles which were then kept in the refrigerator for further phytochemical analysis using the procedures of Sofowora (1993).

Test for alkaloids: About 0.5g of each extract was stirred in 5ml of 1% aqueous hydrochloric acid on a steam bath, allowed to cooled and filtered. 1ml of the filtrate was treated with a few drops of Meyer's reagent and to another 1ml of the filtrate, a few drops of Dragendorff's reagent was added. Turbidity or precipitation with either of the reagents was taken as preliminary evidence fro the presence of alkaloids in the extract being evaluated.

Test for saponins: Frothing test was used to detect the presence of saponins. Approximately 0.5g of each plant extract was mixed and shaken with water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

Test for tannins: Approximately 5g of each portion of the herbal extract was stirred with 10ml of distilled water on a magnetic stirrer, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins.

Test for anthraquinones: Approximately 5g of each herbal extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene. The benzene layer was then separated and 10% ammonia solution was added to half of its volume. A pink, red or violet colouration in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extracts.

Test for cardiac glycosides: Salkoski test was used to identify cardiac glycosides. 0.5g of the extract was dissolved in 2ml of chloroform and sulphuric acid carefully added to form a lower layer. Formation of reddish-brown colour at the interface indicated the presence of steroidal ring (i.e. aglycone portion of the cardiac glycoside).

Test for carbohydrates: Molisch's test was used to detect the presence of carbohydrates. One drop of concentrated sulphuric acid was added to about 1g of the herbal extract, and then three drops of 1% α -naphthol in 80% ethanol were added to the mixture, without mixing to form an upper phase. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates.

Test for flavonoids: Shinoda test was used. To alcoholic solution of the extracts, magnesium powder and few drops of concentrated HCl were added. Formation of orange, pink, red to purple colours indicated the presence of flavonoids (Silva *et al.*, 2003).

Antibacterial susceptibility tests: Clinical Laboratory Institute Standards methods were adopted for the determination of the spectrum of antibacterial activities of the crude herbal extracts (CLIS, 2005). Paper disks were prepared with various concentrations of the methanol extracts from a stock solution (Taura *et al.*, 2004). The dilution formula used was:

$V_1 = R [V_2/O]$ where:

V_1 = initial volume of the solution.

V_2 = final volume of the solution.

R = required concentration.

O = observed concentration.

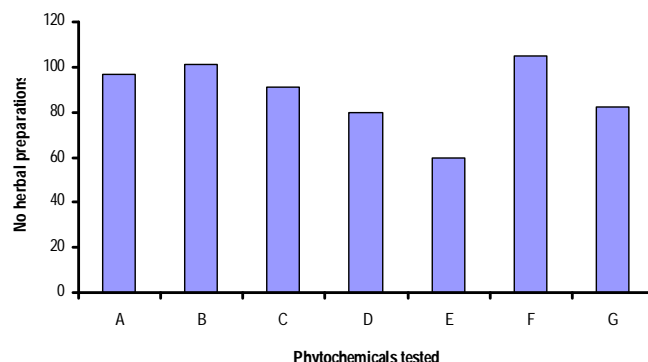
The prepared standard paper disks measuring 6mm in diameter were then sterilized in clean bijou bottles in an autoclave. Standard solutions of the extracts were prepared by weighing approximately 0.8g in 2ml of the initial solvent used for the extraction to get a stock concentration of 400mg/ml. Serial doubling dilution was then employed to obtain five other concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml). One hundred (100) disks were placed in each of the six tubes containing the different concentrations of the herbal extracts until all the extracts were completely absorbed. The impregnated disks were then dried in LTE IP30-UF oven at 30°C for 20 min. Antibacterial activities of the various extracts were then tested on clinical bacterial isolates comprising: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella spp.*

Determination of zones of bacterial inhibition: disk diffusion test using the procedures of the Clinical Laboratory Institute Standards (CLIS, 2005) were employed. Five colonies of bacterial growing in culture were selected and inoculated onto Mueller-Hinton broth and incubated at 35°C for 24hrs. A sterile syringe and needle were used to measure and transfer approximately 0.5ml of the culture to the surface of Mueller-Hinton agar. Using a sterile bent glass rod, the inoculum was spread on the surface of the agar. A sterile pair of forceps was used to place the appropriate extract test disks on the Mueller-Hinton agar medium. The plates were then incubated at 35°C for 18hrs. Then the diameters of the zones of inhibitions were measured to the nearest millimeter using a transparent metric rule.

Determination of the minimum inhibitory concentrations: The conventional tube dilution procedures were employed (Cheesbrough, 2000).

RESULTS

The results of the phytochemical analyses of the medicinal herbal preparations are presented in Fig. 1.



A-Alkaloids, B-Tannins, C-Saponins, D-Flavonoids,
E-Cardiac glycosides, F-Carbohydrates, G-Anthraquinone

FIG. 1. PHYTOCHEMICAL COMPONENTS OF THE HERBAL PRODUCTS COLLECTED FROM DIFFERENT PARTS OF KADUNA METROPOLIS.

Alkaloids were found in 97 (64.7%) of the samples. The corresponding values for tannins, saponins, flavonoids, cardiac glycosides, carbohydrates and anthraquinones were: 101 (67.3%), 91 (60.7%), 80 (53.3%), 60 (40%), 105 (70%) and 82 (54.7%) respectively. The number of methanolic extracts that were able to inhibit the growth of each of the bacterial isolates which signified the presence of antibacterial activities were observed and recorded as positive in Table 1.

Out of 150 methanolic extracts of the herbal medicinal preparations tested, 81 (54%) had activities on *Staphylococcus aureus*, 89 (59.3%) had activities on *E. coli* while 74 (49.3%) and 63 (42%) showed activities on *Salmonella typhi* and *Shigella spp* respectively. The number of herbal preparations showing MICs at various concentrations of their extracts is presented in Table 2. None of the herbal extracts showed their minimum antibacterial activities at the concentration of 12.5mg/ml.

TABLE 1. NUMBER AND PERCENTAGE OF METHANOLIC EXTRACTS OF THE HERBAL PRODUCTS ABLE TO EXERT INHIBITORY EFFECTS ON TEST BACTERIAL ISOLATES.

Bacterial isolates	Number	
	+ve (%)	-ve(%)
<i>Staphylococcus aureus</i>	81(54.0)	69(46.0)
<i>Escherichia coli</i>	89(59.3)	61(40.7)
<i>Salmonella typhi</i>	74(49.3)	76(50.7)
<i>Shigella spp</i>	63(42.0)	87(58.0)

TABLE 2. THE NUMBER OF METHANOLIC EXTRACTS OF HERBAL PRODUCTS WITH MICs IN THE GIVEN CONCENTRATIONS AGAINST TEST BACTERIAL ISOLATES.

Test bacteria	No methanolic extracts with MICs at given conc (mg/ml)					
	400	200	100	50	25	12.5
<i>Staphylococcus aureus</i>	30	22	19	9	1	0
<i>Escherichia coli</i>	33	37	16	3	0	0
<i>Salmonella typhi</i>	29	18	17	9	1	0
<i>Shigella spp</i>	36	19	6	2	0	0

DISCUSSION

The results showed that herbal medicinal products under study contained alkaloids, tannins, saponins, flavonoids, cardiac glycosides, carbohydrates and anthraquinones. Herbal medicinal products are virtually known to contain phytochemicals. These phytochemicals which are the active constituents of plants have been reported to be available from the extracts obtained from many medicinal plants growing in Nigeria (Ajibola & Motoyoshi, 1992).

As natural chemicals they have either antioxidant or hormone-like actions and are promoted for the prevention and treatment of many health conditions, perhaps because they are readily available and could be sourced from various plants/parts such as fruits, vegetables, beans, grains etc, as well as the fact that scientists have identified thousands of them in materials selected for studies because of local uses in traditional medicines (Farnsworth, 1990).

Some of the more commonly known phytochemicals, including flavonoids, carbohydrates, tannins, cardiac glycosides, saponins and anthraquinones were observed in the present study and have all been reported to all aid animals and man by creating a preventive barrier against diseases and sicknesses (Ajibola & Motoyoshi, 1992). Their consistent presence in the herbal products in the present study may be taken to indicate that the products are effective as prescribed by the herbalists. Their varied occurrences in various herbal preparations will however indicate that probably, their therapeutic effect(s) are not the direct effect of a single group

or compound, but rather that the compounds possibly act in combination to bring about an effect.

The rate of microbial resistances to existing therapeutic agents is an increasing serious medical problem. This is presently considered to be of great concern among clinicians and medical microbiologists and is believed to be the common cause of treatment failures in bacterial infectious diseases (Power, 1998). Consequently the observed inhibitory effects of the various herbal extracts in the present study on the tested bacterial isolates is a justification for the need to explore the various traditional modes of diseases treatments in order to determine their various antimicrobial efficacies. This is very important as it will assist in standardizing traditional herbal medicaments. Lack of standardization has been described (Sofowora, 1993) as one of the problems militating against recognition of traditional medicinal practices by orthodox medical practitioners.

With the evidence of antibacterial activities of the methanolic extracts of the herbal preparations under study, it can be rationally suggested that further work needs to be done to identify the chemical natures of the active principles as well as their modes of actions on bacterial cells and their roles in diseases curing. It is also important that more species of pathogenic bacteria be tested in order to ascertain the spectra of activities of the antibacterial substances present in the herbal preparations.

REFERENCES

- Adeleye, I. A. & Opiah, L. (2003). Antimicrobial activity of extracts of local cough mixtures on upper respiratory tract bacterial pathogens. *West Indian Medical Journal*. 52:188-190.
- Ajibola, A. & Motoyoshi, S. (1992). Contribution to the phytochemistry of medicinal plants growing in Nigeria as reported in the 1979 – 1990 literature. A review. *African Journal Pharmacy and Pharmaceutical Sciences*. 22 (3):172-201.
- Awosika, D. F. (1993). Local medicinal plants and health care of consumers. *Clinical Pharmacy and Herbal Medicine*. 9b:28-31.
- Barret, B. (2003). Medicinal properties of Echinaceae: a critical review. *Phytomedicine*. 10(1):66-86.
- Bhushan, P. (2005). Traditional Medicine: A novel approach for available, accessible and affordable health care. Paper for regional consultative on medicine. WHO Regional office for South East Asia.
- Chainani, W. N. (2003). Safety and anti-inflammatory activity of Curcumin: a component of tumeric (*Curcuma longa*). *The Journal of Alternative and Complementary Medicine*. 9 (1):161-168.
- Chen, C. J., Huang, Y. C., Chiu, C. H., Su, L. H. & Lin, T. Y. (2005). Clinical features and genotyping analysis of community acquired methicillin-resistant *Staphylococcus aureus* infections in Taiwanese children. *Journal of Pediatric Infectious Diseases*. 24 (1):40-45.
- Cheesbrough, M. (2000). *District Laboratory Manual for Tropical Countries*. Cambridge University press, U.K.
- Clinical Laboratory Institute Standards (CLIS) (2005). Performance standards for antimicrobial susceptibility. 15th informational supplement (CLIS document M100 –S15). Vol. 25, Wayne, P.A.
- Farnsworth, N. R. (1990). The role of ethno-pharmacology in drug development. In: Chadwick, D.J. and Marsh, J. (Eds.). *Bioactive compounds from plants*. Wiley, N.Y.
- Igile, G. O., Oleszek, W., Jurysta, M. & Burda, S. (1994). Flavonoid veronica amygdaline and their antioxidant activities. *Journal of Agriculture and Food Chemistry*. 432:2445-2448.
- Khan, M. R., Kilara, M. & Omoloso, A. D. (2003). Antibiotic activity of *Couroupita quianensis*: *Journal of Herbs, Spices and Medicinal Plants*. 10 (3):95-108.
- Musa, K. Y., Ahmed, A., Ibrahim, H., Arowosaiye, G. & Olonitola, O. S. (2000). Phytochemical and antimicrobial studies of leaves of *Acalypha racemosa*. *Nigerian Journal of Natural Products and Medicine*. 4:67-69.
- Nkere, C. K. & Iroegbu, C. U. (2005). Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida*. *African Journal of Biotechnology*. 4(6):522-526.
- Pearce, M. C., Fenlon, D., Low, J. C., Smith, A. W., Knight, H. I., Evan, J., Foster, G., singe, B. A., & Gunn, G. J. (2004). Distribution of *Escherichia coli* O157 in bovine faecal parts and its impact on estimates of the prevalence of faecal shedding. *Applied and Environmental Microbiology*. 70:5737-5743.
- Power, E. G. M. (1998). Bacterial resistance to antibiotics. In: Hugo, W.B. and Russell, A. D. (ed.). *Pharmaceutical Microbiology* (6th edn). Blackwell sciences.
- Silva, G. L., Lee, I. & Kinghorn, A. D. (2003). Natural products isolation. In Cannel, R. J. P. (Ed.). *Methods in Biotechnology*. Human Press Inc., Totowa, N.J.
- Sofowora, A. (1993). *Medicinal plants and Traditional Medicine in Africa*. Spectrum Books Ltd (Pub.), Ibadan.
- Taura, D. W, Okoli, A. C. & Bichi, A. H. (2004). In-vitro activities of ethanolic extracts of *Annona cosmosus* L., *Allium sativum* L. and *Aloe barbadensis* L. in comparison with ciprofloxacin. *Biological and Environmental sciences Journal for the Tropics*. 1(1):36-41.