Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 3 (1): 23-28, 2009
ISSN 1819-3455
© 2009 Academic Journals Inc.

Comparative Study of the Ethanolic Extracts of Four Nigerian Plants Against Some Pathogenic Microorganisms

[1]J. Nebedum, [2]K. Ajeigbe, [3]E. Nwobodo, [1]C. Uba, [4]O. Adesanya,
[2]O. Fadare and [4]D. Ofusori
[1]Department of Biochemistry, Faculty of Medicine,
Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Physiology, School of Basic Medical Sciences, Igbinedion University,
Okada, Benin-City, Nigeria
[3]Department of Physiology, Faculty of Medicine,
Nnamdi Azikiwe University, Nnewi, Nigeria
[4]Department of Anatomy, School of Basic Medical Sciences, Igbinedion University,
Benin City, Nigeria

Abstract: The ethanolic extracts of *Cassia alata* (CA), Walnut-*Juglan nigra* (JN), *Ocimum basilicum* (OB) and *Aloe vera* (AV) were studied for their *in vitro* antimicrobial activity against tested pathogenic microorganisms using Agar diffusion method. Preliminary phytochemical screening showed the presence of tannin, fats and oil, saponins and glycosides in the ethanolic extracts of all tested plants. *Juglan nigra* has the highest activity against all tested organisms *Escherichia coli*, *Staphylococcus aureus* and *Candida albican*. While the least activity against tested organism was shown by OB, ethanolic extracts of AV was the most effective against *Staphylococcus aureus*, while JN was the most effective against *Escherichia coli* and *Candida albican*. Also, the combined 600 mg mL^{-1} (concentration) of the four extracts showed a remarkable inhibitory effect on the organisms; produces over 50% of the activity of a standard antibiotic, Fulcin. Walnut-*Juglan nigra* (JN) showed the best antibacterial activity out of the four; hence this plant can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

Key words: Pathogenic microorganisms, antimicrobial activity, *Juglan nigra*, *Ocimum basilicum*, *Cassia alata*, *Aloe vera*

INTRODUCTION

Medicinal plants are a source of great economic value in the African continent (Iwu, 1993). The ethnomedicinal resources of Africa remain largely unexploited. Medicinal plants can be described as nature pharmacy for nearly 80% of people living in Africa (WHO, 2002). Nature has bestowed on us a very rich botanical wealth and a large number of diverse plants growing in different part of the continent. In Nigeria, thousands of species are known to have medicinal value (Sofowora, 1982) and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Okwu and Josiah, 2006; Rios and Recio, 2005). Because of better cultural acceptability and fewer side effects herbal medicine still remains the mainstay of 75-80% of the whole population in the developing countries for primary health care (Ghasi *et al*., 2000). However, the last few years have seen a major increase in their use in the developed world (Dahanukar *et al*., 2000). Nowadays

Corresponding Author: Ajeigbe, Kazeem Olasunkanmi, Department of Physiology, School of Basic Medical Sciences, Igbinedion University, Okada, P.M.B. 0006, Benin City, Nigeria Tel: +234 803 570 5220

multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial and chemosynthetic drugs for the treatment of infectious diseases (Nascimento *et al*., 2000). In addition to this problem antibiotics are sometimes associated with adverse effects on host including hypersensitivity, immune suppression and allergic reactions (Wink, 1999). This situation may have motivated scientists to search for new alternatives to antimicrobial and chemosynthetic drugs, which has been found in herbs without the above mentioned side effects.

The use of plants extractives for therapeutic purposes dates back to the earliest times. Accounts of application of medicinal plants by local traditional Nigerian doctors in the preparation of a wide variety of medicaments abound (Akah, 1994).

There is need to continually explore this rich resource for possible exploitation by scientifically validating the folkloric claims. This will serve two important purposes: to discover candidate drugs of natural origin for development and to justify their continued administration to human patients in this part of the world. Such scientific evaluations will help to establish the safety margin in terms of dosage, toxicity and side effects.

In this study, we investigated the much claimed antimicrobial activity of four Nigerian plants extractives on three common microorganisms. The goal of the study was primarily not only to scientifically validate the claims but to establish their efficacy.

MATERIALS AND METHODS

Plants Collection and Preparation

The plants used in this study were *Cassia alata* (CA), Walnut-*Juglan nigra* (JN), *Ocimum basilicum* (OB) and *Aloe vera* (AV). They were collected from the clearings of Nsukka and Awka, Nigeria, in June 2007. The plants specimens were identified and authenticated at the herbarium of the University of Nigeria, Nsukka where voucher specimens were deposited. The plants grow wild in the forest clearing of West Africa. All had been taxonomically described and classified (Githens, 1948). Large quantities were air-dried for nine days. Electric blender was used to grind them into powder which was extracted in 70% ethanol using soxhlet extractor. The extract was later heated to dryness in a water bath at approximately 55°C so as to get rid of the ethanol. The yield was about 6 g (w/v).

Phytochemical Screening

Phytochemical screening was conducted for the different extracts so as to ascertain the major constituents of their leaves. Standard protocols were employed (Trease and Evans, 1983; Goodwin and Mercer, 1983). The phytochemistry of most of the plants had been previously described (Gundidza, 1985).

Preparation of Nutrient Agar Plates

About 8.5 g of nutrient agar was dissolved in 300 mL of distilled water. The resulting mixture was properly shaken and sterilized by autoclaving at 121°C at 15 lbs inch^{-2} for 15 min. The Petri-dishes were similarly sterilized. The mixture was allowed to cool and poured into sterilized Petri dishes and then allowed to solidify.

Preparation of Pure Culture of Microorganisms

Specimens of *Staphylococcus aureus*, *Escherichia coli* and *Candida albican* were obtained from Glanson Medical Laboratory, Awka, Nigeria and the Nnamdi Azikiwe University Teaching Hospital at Nnewi, Nigeria. The organisms were collected into bottles containing nutrient broth. Sterilized wire loop was used to inoculate the organisms into petri-dishes containing the media using the Pour Plate method. Briefly, the inoculums was seeded into nutrient agar and properly mixed. This was poured

into Petri dishes and allowed to solidify. Using sterilized glass tube; wells were made on agar plates that were filled with different concentrations of the plant extract. At the end the culture period, the zones of inhibition as observed for the respective dishes were measured.

Antimicrobial Activity of Different Doses of the Plants Extracts

Six grams of stock for each extract was dissolved in distilled water to obtain four concentrations of 600, 300, 60 and 6 mg mL^{-1}. The activity of these concentrations was tested on the three selected microorganisms. Also, following the establishment of anti-microbial activity for the respective extracts; leaf extracts that showed high anti-microbial activity, or greater zones of inhibition were compounded into soap and ointments. The soap and ointment were used for the treatment of randomly selected superficial/ skin infections of volunteers.

Soap-Extract Compounding

One liter of caustic soda was dissolved in 5 L of water and was allowed to stand for 24 h after which the specific gravity of the solution was obtained using a hydrometer to slurry the mixture. Six grams of the plant extract was added which comprises of two grams of AV, 2 g of JD, 1 g of OB and 1 g of CA extracts. Eight grams of silicate was also added to harden the mixture. This mixture was stirred very well, until it developed a hard consistency. This was quickly transferred into the mould and was allowed to set for about 6 h, after which it was removed and was ready for use. The method employed generally followed the ethnomedical method of preparation of topical medicines.

Preparation of Ointments from the Extracts

The 100 g of commercial petroleum jelly and 10 g of candle wax were put in a bowl placed in a water bath to melt. 0.1 g of methyl paraben and 25 g of paraffin oil were added. Two grams of lanolin was used to effect lubrication. Eight grams of the extract mixture that comprised of 3 g of AV, 3 g of JN, 1 g of OB and one gram of CA were added. The mixture was then poured into a small plastic container and was allowed to solidify. Fifteen volunteers who had different kinds of skin infections were chosen for treatment with the ointment. Application of the ointment was topical and for 2-5 times daily for 7 consecutive days. All the volunteers were requested to report back with their assessment of the preparation and for the confirmation.

Treatment of Superficial and Skin Infection of Volunteers

Twenty five volunteers who were undergraduates experiencing different kinds of superficial/skin infections and who were not on any other medication were recruited for this study. The skin infections included skin eruptions, acne, eczema, irritations and insect bites. There was no clinical characterization of the infections. Volunteers were required to use the soap for between 2-5 times daily for seven consecutive days. All the volunteers were requested to report back with their assessment of the preparation and for the confirmation.

RESULTS AND DISCUSSION

The phytochemical screening of the different plants show the presence of some chemical constituents of the leaves: tannins, fats/oil, saponin and glycoside (Table 1). The tests were only qualitative as described earlier (Trease and Evans, 1983).

Table 2-5 explain the inhibitory effects of different concentrations of the various plants on *Escherichia coli*, *Staphylococcus aureus* and *Candida albican*. At 600 mg mL^{-1} concentration, JN has the highest zone of clearing on *C. albican* and *E. coli* while AV has its highest zone on *S. aureus*.

Table 1: Major chemical components of the plants extracts

Plants/Constituents	Tannins	Fats/oil	Saponins	Glycosides
Juglan nigra	+	+	+	+
Ocimum basilicum	+	+	+	+
Aloe vera	+	+	+	+
Cassia alata	+	+	+	+

+: present.

Table 2: *In vitro* antimicrobial activities of 600 mg mL^{-1} extracts of AV, OB, CA and JN on pathogenic microorganisms

	Micro-organisms/Zone of inhibition (mm)		
Extracts	*Candida albican*	*Staphylococcus aureus*	*Escherichia coli*
Aloe vera	1.90	1.82	1.70
Ocimum basilicum	1.60	1.00	0.80
Cassia alata	1.25	1.40	1.60
Juglan nigra (Walnut)	2.10	1.60	1.80

Table 3: *In vitro* antimicrobial activities of 300 mg mL^{-1} extracts of AV, OB, CA and JN on pathogenic microorganisms

	Micro-organisms/Zone of inhibition (mm)		
Extracts	*Candida albican*	*Staphylococcus aureus*	*Escherichia coli*
Aloe vera	0.60	1.63	1.50
Ocimum basilicum	0.83	0.80	0.72
Cassia alata	0.75	1.20	1.50
Juglan nigra (Walnut)	1.80	1.30	1.70

Table 4: *In vitro* antimicrobial activities of 60 mg mL^{-1} extracts of AV, OB, CA and JN on pathogenic microorganisms

	Micro-organisms/Zone of inhibition (mm)		
Extracts	*Candida albican*	*Staphylococcus aureus*	*Escherichia coli*
Aloe vera	0.70	1.00	0.85
Ocimum basilicum	0.30	0.35	0.20
Cassia alata	0.30	0.70	0.80
Juglan nigra (Walnut)	0.70	0.50	0.80

Table 5: *In vitro* antimicrobial activities of 6 mg mL^{-1} extracts of AV, OB, CA and JN on pathogenic microorganisms

	Micro-organisms/Zone of inhibition (mm)		
Extracts	*Candida albican*	*Staphylococcus aureus*	*Escherichia coli*
Aloe vera	0.40	0.30	0.00
Ocimum basilicum	0.20	0.10	0.00
Cassia alata	0.30	0.20	0.20
Juglan nigra (Walnut)	0.40	0.15	0.35

Table 6: Effect of Fulcin and the combined 600 mg mL^{-1} concentrations of the crude extract on the microorganisms

	Micro-organisms/Zone of inhibition (mm)		
Treatments	*Candida albican*	*Staphylococcus aureus*	*Escherichia coli*
Fulcin	5.00	3.21	3.60
Combined 600 mg mL^{-1}	2.82	2.60	2.30

However, CA and OB have their lowest inhibitory effect on *C. albican* and *E. coli* (Table 2). In the same vein, at 6 mg mL^{-1} concentration, JN and AV have their highest zone of clearing on *S. aureus* and *E. coli*, respectively (Table 5). In general, the results show a dose dependent inhibitory effect on the three organisms.

Table 6 shows a comparison of the effects of Fulcin and the combined 600 mg mL^{-1} (concentrations) of the four extracts on the microorganisms. Fulcin was given in dose range of 2 mg mL^{-1} (normal human dose range is 20-30 mg/kg/day). The combined extract produced about 56%

zone of clearing compared to the standard antibiotics-Fulcin used on *Candida albican*, 81% zone of clearing with *Staphylococcus aureus* while it produced 64% zone of clearing with *Escherichia coli*. In essence, *Staphylococcus aureus* is the most susceptible to the combined extracts.

Fifteen of the twenty-five treated volunteers (60%) reported had significant beneficial result. The ointment from the extract and soap showed significant improvement in the 60% of the volunteers. The cream and soap exhibited high antibacterial activity against common skin pathogens, which are usually caused by yeasts and *Staphylococci*. This antimicrobial activity against yeast-*Candida albican* and bacteria-*Staphylococci* is supported by the result. Some 30% requested for more of the either medicaments.

A successful prediction of botanical compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent. Earlier studies have found that plant extracts in organic solvent provided more consistent antimicrobial activity compared to those extracted in water (Parekh *et al*., 2005; Ahmad *et al*., 1998). In this study ethanol extract of four plants were tested against three pathogenic micro-organisms (two bacterial and one yeast) using the agar disc and agar well diffusion.

Ethanol extracts from AV, OB, CA and JN presented antimicrobial activity to all tested micro-organisms at doses above 6 mg mL^{-1}, ethanol extracts from JN and AV presented the highest activities as indicated above. Besides, *C. albican* is very sensitive to all extracts at all doses. This susceptibility of yeast to different plant extracts has been documented (Ahmad *et al*., 1998; Nascimento *et al*., 2000).

E. coli, which is already known to be multi-resistant to drugs (Dromigny *et al*., 2005), also showed partial resistant to extracts of AV, OB at low dose of 6 mg mL^{-1} with 0 cm zone of clearing on agar disk. This microorganism is known to be resistant to different antibiotics, but had its growth inhibited by other extracts of CA and JN.

Researchers have documented the antimicrobial activities of AV (Agarry *et al*., 2005), OB (Luis Carlos Silva *et al*., 2005), CA (Sakharkar and Pati, 1998), JN (Foster and Duke, 1990).

The findings of this study is not contradictory to the earlier reports and hence underscore the possibility to exploit the antimicrobial properties inherent in the AV, OB, CA and JN for large scale medicinal uses. The finding is beneficial as it also heralds probably the emergence of a new antibiotic with such a wide spectrum of activity, as found in the present study. Nevertheless, detailed chemical studies followed by pharmacological investigations and toxicity evaluations are still required to isolate the active principle(s) of these plants.

CONCLUSION

In conclusion, the results showed that OB, JN, AV and CA are potential antifungal and antibacterial agent for the treatment of skin (*Candida albican*) and GIT, urinary tract infections (*E. coli*). Herbal therapies are generally available and cheaper and offer important alternatives to the more expensive conventional medicines.

REFERENCES

Agarry, O.O., M.T. Olaleye and C.O. Bello-Michael, 2005. Comparative antimicrobial activities of *Aloe vera* gel and leaf. Afr. J. Biotechnol., 4: 1413-1414.

Ahmad, I., Z. Mehamood and F. Mohammad, 1998. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol., 62: 183-193.

Akah, P.A., 1994. Abortifacient activity of some Nigerian medicinal plants. Phytother. Res., 8: 106-108.

Dahanukar, S.A., R.A. Kulkarni and N.N. Rege, 2000. Pharmacology of medicinal plants and natural products. Indian J. Pharmacol., 32: 81-118.

Dromigny, J.A., P. Nabeth, A. Juergens-Behr and J.D. Perrier Gros-Claude, 2005. Risk factors for antibiotic resistant *E. coli* isolated from community-acquired urinary tract infections in Dakar, Senegal. J. Antimicrobial Chemother., 56: 236-239.

Foster, S. and J.A. Duke, 1990. Black Walnut in Medicinal Plants. 1st Edn., Houghton Mifflin Co., New York, pp: 276.

Ghasi, S., E. Nwobodo and J.O. Ofili, 2000. Hypocholesterolemic effect of crude leaf of *Moringa oleifera* in high fat diet fed wistar rats. J. Ethnopharmacol., 69: 21-25.

Githens, T.S., 1948. Drug plants of Africa. African Handbooks, 8: 59-59.

Goodwin, T.W. and E.I. Mercer, 1983. An Introduction to Plant Biochemistry. 2nd Edn., Pergamon, Oxford.

Gundidza, M., 1985. Cytochemical screening of some Zimbabwean medicinal plant. The Central Afr. J. Med., 31: 238-239.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. 1st Edn. CRC Press Inc., Florida. ISBN: 0-84934-266-X pp: 239-239.

Luis Carlos Silva, Nubraska Ramírezy, Alfonso J. Rodríguez Morales, 2005. Treatment of giardiasis with *Ocimum basilicum*. Acta Científica Estudiantil., 3: 46-48.

Nascimento, G.G.F., L. Juliana, C.F. Paulo and L.S. Giuliana, 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian J. Microbiol., 31: 247-256.

Okwu, D.E. and C. Josiah, 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotechnol., 5: 357-361.

Parekh, J., D. Jadeja and S. Chanda, 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk. J. Biol., 29: 203-210.

Rios, R.L. and M.C. Recios, 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol., 100: 80-84.

Sakharkar, P.R. and A.T. Pati, 1998. Antimicrobial activity of *Cassia alata*. Indian J. Pharm. Sci., 60: 311-312.

Sofowora, E.A., 1982. Medicinal Plants and Traditional Medicinal in Africa. 1st Edn., University of Ife Press, Nigeria, pp: 1-23.

Trease, G.E. and I.C. Evans, 1983. Textbook of Pharmacognosy. 12th Edn., Bailliere Tindall, London, pp: 21-22.

WHO, 2002. Traditional medicine strategy launched. In Bulletin of the World Health Organization. Bull. World Health Org., 80: 610-610.

Wink, M., 1999. Introduction to Biochemistry, Role and Biotechnology of Secondary Products. In: Biochemistry of Secondary Product Metabolism, Wink, M. (Ed.). CRC Press, Boca Raton, FL., pp: 1-16.