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Methods of Extraction and Antimicrobial Susceptibility Testing of Plant Extracts

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Abstract: Plant extracts are widely used in pharmaceutical, food and cosmetic industry; however the effect of conventional methods of extraction described in this paper as cold and soxhlet method has not been investigated. Antimicrobial activity of *Anchomanes difformis* (Blume) Engl. leaf extracts have been described comparing cold and soxhlet method of extraction. The amount of extractable plant extract/phytochemicals, the solubility of the extracts and the susceptibility of the test organism to the extract had a correlation with the extraction methods. Cold extract had wider zones of inhibition and activity than soxhlet extract. Though there was no significant difference ($P>0.05$) between cold and soxhlet extracts on the entire test organisms, differences exist when compared individually. This paper revealed cold method of extraction as a better alternative to soxhlet method in antimicrobial susceptibility assay. However, the decision on the methods of extraction to be used could also be dependent on the amount of certain phytochemicals required and the solubility of the extracts in solvents after extraction.

Key words: Extraction methods · Plants · Antimicrobial

INTRODUCTION

Majority of people on this planet relies on *materia medica* (medicinal plants and other materials) for their everyday health needs. 80% of the world population relies on plant derived medicine for their health care [1, 2]. Plant derived medicine can be a base for the development of new drugs [3]. Plants contain a broad range of bioactive compounds such as lipids, phytochemicals, pharmaceutics, flavors, fragrances and pigments. Plant extracts are widely used in the food, pharmaceutical and cosmetics industries. Extraction techniques have been widely investigated to obtain such valuable natural compounds from plants for commercialization. However, the effect of conventional methods of extraction: Soxhlet extraction method [4-7] and cold methods [8-9] on antimicrobial assay has not been documented. This paper investigated the possible effect of these methods of extraction on antimicrobial susceptibility testing of plant using *Anchomanes difformis* (Blume) Engl. as a case study to maintain an appropriate standard of extraction method in antimicrobial assay. Lack of such standard could lead to different results on antimicrobial assay making it difficult to reproduce research work.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: Fresh leaves of *A. difformis* were collected from Mesan, A village around Atan in Adodo-Ota Local Government of Ogun State, Nigeria. The plant was identified as *A. difformis* (Blume) Engl. (family = Araceae) in the department of Biological Sciences, Covenant University, Canaanland, Ota, Ogun State, Nigeria.

Preparation of the Plant Extract: The leaves were dried to a constant weight at 18°C in an enclosed air conditioned research laboratory. The dried leaves were blended to powder to increase the surface area for extraction and divided into two equal parts for both soxhlet and cold extraction procedures. The entire weight of the leaves powder was 100g.

Methods of Extraction: Two methods of extraction were analyzed in this study: Soxhlet method of extraction and cold method of extraction. In a conventional Soxhlet system, plant material is placed in a thimble-holder and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and

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unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved [10]. The powdered leaves were extracted with the appropriate volume of solvent at 80°C for 8h [11]. Cold extraction was carried out by soaking the powdered leaves for 72h in an enclosed glass jar. Solvent used for both extractions was a mixture of ethanol, methanol and water in the ratio of 70:20:10. Equal volume of solvent in the same ratio was used for both methods of extractions. The solvents were letter separated from the extract with the aid of rotary evaporator at 40°C.

Concentration of the Extract: Varying weight (0.3g, 0.2g and 0.1g) of the extract was weighed and dissolved individually in 1ml of DMSO to give concentrations of 0.3g/ml, 0.2g/ml and 0.1g/ml respectively. These concentrations apply to both cold and soxhlet extract.

Test Organism: The test organisms were carefully selected microorganisms including a number of Gram-positive, Gram-negative and yeast that were known to be of clinical and public health importance. These test organisms include: *Salmonella typhi, Staphylococcus aureus, Proteus vulgaris, Shigella flexneri 004, Shigella dysenteriae 006, Escherichia coli, Escherichia coli* NCTC 12900, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Salmonella paratyphi, Pseudomonas aeruginosa* and *Candida albicans*. All these test organisms were obtained as pure isolates on agar slant from Microbiology and Biochemistry Departments of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos and were aseptically subcultured into nutrient broth and incubated for 6-8 hours to ensure that the organisms were at their exponential phase of growth before carrying out the sensitivity analysis.

Antimicrobial Assay: The organisms in the nutrient broth were serially diluted using sterilized distilled water (sterile water) and the 10^{-7} dilution corresponding to 0.5 MacFarland standard were used as the inoculum. Mueller Hinton Agar (MHA) for bacteria and Saubouraud Dextrose Agar (SDA) for yeast were used. Broth media were measured and dissolved in appropriate volume of distilled water, following the manufacturer's guideline; and was sterilized by autoclaving. Pour plate techniques was used; about 1ml of the standardized inoculum was mixed with the medium in a sterile container to ensure that the test organisms were evenly distributed and poured into sterile petridishes and allowed to set. Each plate contains equal volume of the media. The antibacterial activity of the crude extracts was determined in accordance with the standard agar-well diffusion method [12].

The plates were incubated at 37°C for bacteria and 28°C for yeast. The plates were observed for zones of inhibition after 24 h for bacteria. It was observed that the yeast also was able to produce discrete colonies within 24 hours, thus all the plates were read after 24hours.

The plates were inoculated with the same standardized inoculum to check for the viability of the organisms as organism viability control. This implies that any clear zone of inhibition observed is due to the activity of the extract. All the organisms showed viability with colonies covering 100% surface of the plate. The second control is to test the activity of the solvent (DMSO) used to dissolve the extract to ensure that the activity is not due to action of the solvent on the test organisms. The solvent showed zero activity on all the test organisms.

Plates were read by measuring observed clear zones (area without growth) of inhibition around the wells containing plant extract. Measuring rule in millimeter was used to take the measurement from the edge of the well to the end of the clear zone of inhibition. No measurement was taken if no clear zone of inhibition was observed.

The estimation of MIC of the crude extracts was carried out by standard method [13].

Phytochemical Analysis: Phytochemical test were carried out on the extract of the powdered leaves from soxhlet and cold extraction, using standard procedures [14-17]. Quantitative assay of total phenol was determined spectrophotometrically. Note that all chemicals used were freshly prepared.

RESULTS

It was observed that the cold leaf extract was soluble in water after vigorous shaking and forms homogenous clear solution on heating. The soxhlet extract was insoluble in water, but later became partially soluble after vigorous shaking. Soxhlet extract was very soluble in petroleum ether and the solubility increases on heating. Cold extracts were poorly soluble in petroleum ether.

Table 1: Semi-quantitative phytochemical analysis of the leaves extract

Phytochemical Components	Cold extraction Leaf	Soxhlet extraction Leaf
Tannins	+++	++
Phlebotannins	-	-
Steroids	+	+++
Terpenoids	-	-
Cardiac glycosides	+++	+
Glycosides	-	-
Saponins	+++	+++

+ = Positive, - = Negative

Table 2: Quantitative Amount of Phytochemical Components

Phytochemical Components	Cold extraction Leaf	Soxhlet extraction Leaf
Alkaloids	12.4%	16.0%
Flavonoids	94.0%	63.0%
Tannins	92.8%	92.6%
Total Phenol	75.0%	76.0%

Table 3: Zones of Microbial Inhibitions by Root and Leaf Extracts of *Anchomanes difformis* (Blume) Engl

	Cold extraction Leaf			Soxhlet extraction Leaf		
Organisms	0.1 g/ml	0.2 g/ml	0.3 g/ml	0.1 g/ml	0.2 g/ml	0.3 g/ml
Salmonella paratyphi	4.1*	5.2	5.9	2.9	4.1	5.0
Salmonella typhi	2.2	2.3	2.4	0.9	2.1	2.9
Candida albicans	4.1	5.2	5.4	0.9	2.0	3.1
Proteus vulgaris	1.0	2.0	2.1	1.0	1.8	2.0
Staphylococcus aureus	3.0	4.9	5.3	5.7	5.8	6.1
Shigella flexneri	0.0	0.0	0.0	0.0	0.0	0.0
Shigella dysenteriae	4.0	4.3	4.6	2.0	2.1	2.3
Escherichia coli (wild strain)	1.0	2.0	2.1	1.0	2.9	4.1
Escherichia coli (NCTC 12900)	1.0	1.9	3.8	0.0	0.0	0.0
Escherichia coli ATCC 35218	3.0	6.1	7.0	3.9	4.0	4.2
Escherichia coli ATCC 25922	5.0	5.0	5.0	4.0	4.0	4.0
Pseudomonas aeruginosa	2.1	4.0	5.3	0.0	0.0	0.0

* Zone of inhibition given in mm

Both cold and soxhlet leaf extract were partially soluble in ethanol and methanol with increased solubility on shaking. All the extracts were very soluble in dimethysulfoxide (DMSO) after vigorous shaking. The soxhlet extract was oilier than the cold extract. Cold leaf extract was greenish-brown in colour, while the soxhlet leaf extract was greenish in colour. More extract were obtained from soxhlet extraction than cold extraction: 50g of powdered leaves yielded 9.99g of extract by soxhlet extraction method, while 50g of powdered leaves in cold extraction method yielded 5.99g of the extract. Phytochemical analysis showed the same number of phytochemicals, but in varying amount. Cold extract contains more tannins, cardiac glycosides and flavonoids while soxhlet extract contains more phenol, alkaloids and steroids (Tables 1 and 2). Though the effect of the extract on the entire organism was not significant ($p>0.05$) differences exist when compared individually (Table 3). Zones of inhibitions were generally wider at MIC (0.1g/ml) and other concentration with cold extract than soxhlet extract (Table 3). *Pseudomonas aeruginosa* and *Escherichia coli* NCTC12900 were resistant to the soxhlet extract but susceptible to the cold extract (Table 3).

DISCUSSION

Methods of extraction can affect the physical properties of the extract, especially its solubility in solvents. Differences in solubility could be as a result of heat associated with the soxhlet extraction method [10]. This had also shown that solvent to be used in the reconstitution of the extract should be considered before adopting a particular extraction methods as it could affect the solubility of the extract in a solvent. The soxhlet extract were however more oilier than the cold extract [18]. Most of the soxhlet extracts were oily in nature

suggesting that soxhlet extraction could be one of the novel methods of extracting oil from leaves. Soxhlet extraction yielded more extract than cold extraction, indicating that it is a better method of obtaining more extractable component from leaves. Phytochemical analysis had revealed that though the same number of phytochemicals can be recovered from both extracts, there varies in their amount, higher amount of phenol, alkaloids and steroids in soxhlet extract had revealed that these phytochemicals are better recovered from leaves by the application of heat, while better recovery of tannins, cardiac glycosides and flavonoids is by cold extraction. Lower zones of inhibition as observed with soxhlet extract compared to cold extract could be due to inactivation of thermolabile components of the extract by heat [19]. *P. aeruginosa* and *E. coli* NCTC12900 resistance to soxhlet extract could still be probably due to the prolonged effect of heat on the extract as the same phytochemicals were present in both extract, but the action of heat in soxhlet extract had inactivated some active components of the extract. Heat that is usually associated with soxhlet extraction method [10] accounts for the differences observed in this antimicrobial susceptibility testing. In conclusion, it was shown that wider zones of inhibition were obtained with cold extraction than soxhlet extraction with certain organism capable of being resistant to soxhlet extract. Also, the amount of the extracts and the various phytochemicals and the nature/solubility of the extract could vary with the method of extraction.

CONCLUSION

In conclusion, the solubility of extract, amount of a particular phytochemicals (Table 1) and the total amount of extract varies with the extraction method used in this research. Cold method of extraction produced wider zones of inhibition and more activity, indicating that cold method could be better than soxhlet method in antimicrobial assay of *Anchomanes difformis* (Blume) Engl. Using the solvent reported above.

REFERENCES

1. WHO, 2002, WHO Traditional Medicine Strategy 2002-2005, Geneva, pp: 1-63.
2. Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine, 27: 1-93.
3. Iwu, M., 1993. Handbook of African medicinal plants. CRC Press, Boca Raton, FLe.
4. Agyare, C., A.Y. Mensah and S.O. Asante, 2006. Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana. Boletin latinoamericano ydel caribe de plantas medicinales Y Aromaticas Noviembie, l5(006): 113-117.
5. Makut, M.D., S.D. Gyar, G.R.I. Pennap and P. Anthony, 2008. Phytochemical Screening and Antimicrobial Screening of the ethanolic and methanolic extracts of the leaf and bark of *khaya senegalensis*. African J. Biotech., 7(9): 1216-1219.
6. Reuben, K.D., F.I. Abdulrahman, J.C. Akan, H. Usman, O.A. Sodipo and G.O. Egwu, 2008. phytochemical screening and invitro antimicrobial investigation of the methanolic extract *Croton zambesicus* muell ARG. Stem bark. European J. Scientific Res., 123(1): 134-140.
7. Awobajo, F.O., I.I. Olatunji-Bello, O.A. Adegoke and T.O. Odugbemi, 2009. phytochemical and antimicrobial screening of *Hybanthus Enneaspermus* and Paquetina Nigricense, 1(4): 159-160.
8. Ayoola, G.A., H.A.B. Cooker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweya, E.C. Ezennia and T.O. Atangbayila, 2008. phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Tropical J. Pharmaceutical Res., 7(3): 1019-1024.
9. Igbinosa, O.O., E.O. Igbinosa and O.A. Aiyegoro, 2009. Antimicrobial action and phytochemical screening of stem bark extract from *Jatropha curcas* (Linn). African J. Pharmacy and Pharmacol., 3(2): 056-062.
10. Wang, L. and C.L. Weller, 2006. Recent Advances in extraction of Nutraceuticals from plants. Trend in food science and technology (Elsevier), 17: 300-312.
11. Akroum, S., D. Satta and K. Lalaoui, 2009. Antimicrobial, Antioxidant and Cytotoxic Activity and Phytochemical screening of some Algerian Plants. European J. Scientific Res., 31(2): 289-295.
12. Irobi, O.N., M. Moo-Young, W.A. Anderson and S.O. Daramola, 1994. Antimicrobial activity of the bark of *Bridelia ferruginea* (Euphobiaceae). Intern. J. Pharmacog, 34: 87-90.
13. Kinpelu, D.A. and D.O. Kolawole, 2004. Phytochemical and antimicrobial activity of leaf extract of *Piliostigma aaaathonningii* (Schum). Sci. Focus J., 7: 64-70.

14. Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa (2nd edn.). Spectrum Books Ltd, Ibadan, Nigeria, pp: 9-25.
15. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy (11th edn.). Braille Tirida Canada Macmillan Publishers, Canada, pp: 257.
16. Harbone, J.B., 1973. Phytochemical methods. Chapman and Hall Ltd. London, pp: 49-188.
17. Boham, A.B. and A.C. Kocipai, 1994. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccininum vaticulum* and *vicalycinium*. Pacific Sci., 48: 58-463.
18. Hanmoungjai, P., L. Pyle and K. Niranjan, 2000. Extraction of rice bran oil using aqueous media. J. Chemical Technol. and Biotechnol., 75: 348-352.
19. Luque-Garcia, J.L. and M.D. Luque De Castro, 2004. Ultrasound-assisted Soxhlet extraction: An expeditive approach for solid sample treatment-Application to the extraction of total fat from oleaginous seeds. J. Chromatography A., 1034: 237-242.