Egypt. J. Exp. Biol. (Bot.), 13(2): 181 – 186 (2017)

DOI: 10.5455/egyjebb.20170509072122

RESEARCH ARTICLE

Samuel, Temitope Oluwaseun
Egwuatu T.O.G
Ajose F. O.

Effect of drying methods on the antimicrobial properties of *Cassia alata, Commelina diffusa* and *Borreria verticillata* extracts

ABSTRACT:

This study investigated the effect of different drying methods (Sun dry; Shade dry, 40ºC Oven dry and 60ºC Oven dry respectively) on the antimicrobial activities of leaves extracts of *Borreria verticillata, Cassia alata* and *Commelina diffusa.* Using disc diffusion agar method, the antimicrobial assay of the methanol and aqueous extracts of each plant leaves at different drying methods were carried out. The results from the study revealed that drying methods affect the efficacy of the tested medicinal plants by influencing the quantity and quality of the bioactive constituents present in the plants. It was concluded from the study that aqueous methanolic crude leaves extract dried via shade-dry and 40 ^{0}C oven dry methods is most effective so, the used drying methods are suitable to employ for processing plants for use. This was proved as these (shade-dry and 40ºC) methods retained most of the bioactive constituents of the plants thereby inhibited the growth of the tested microbes.

KEY WORDS:

Antimicrobial properties, Fungi, Bacteria, Leaves extract.

CORRESPONDENCE:

Samuel, Temitope Oluwaseun

Department of Botany, Faculty of Science, University of Lagos, Nigeria.

E-mail: tosamuel@unilag.edu.ng

[1] Egwuatu T.O.G

[2] Ajose F. O.

[1] Department of Microbiology, Faculty of Science, University of Lagos, Nigeria.

[2] Dermatology Units, College of Medicine, Lagos State Teaching Hospital, Nigeria.

ARTICLE CODE: 19.02.17

INTRODUCTION:

Plants with health-promoting characteristics, relief from symptomatic problems and have curative properties are generally referred to as medicinal plants (Sonibare and Abegunde, 2012). Plant extracts have been used traditionally to treat several infectious diseases including those caused by bacteria, fungi, protozoa and viruses (Sadeghi-Nejad and Deokule, 2009). The physical and chemical properties of medicinal plants are determined by their moisture content. The first step in many post-harvest operations is removal of water that is, drying. According to Oztekin and Martinov (2007), drying is defined as the decreasing of plant moisture content, aimed at preventing enzymatic and microbial activity, and consequently preserving the product for extend shelf life. As a result of this, adequate drying is required to provide a rapid reduction in the moisture content without affecting the quality of the active ingredients of medicinal plants. Medicinal plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms or buildings; by direct sunlight, if appropriate; in drying ovens and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. When possible, temperature and humidity are controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of colour of leaves and flowers; and lower temperatures are employed in the case of medicinal plant materials containing volatile substances (Farias, 2003).

Borreria verticillata Linn, belongs to the family Rubiaceae and has been reported to have several uses in herbal medicine (Burkill, 2000). *Cassia alata* Linn, also known as the Candle Bush is a flowering plant, as well as medicinal plant. This ornamental shrub belongs to the family Fabaceae.

Various parts of this plant have been used in herbal medicine (Mohideen *et al.,* 2005). *Commelina diffusa* Burm. f., otherwise known as the climbing dayflower is a perennial herbaceous plant in the family Comelinaceae. *C. diffusa* is a troublesome weed which according to Edmunds (1971) was once encouraged as a ground cover to mitigate soil erosion. The leaves infusion is used as a wound-healing agent in traditional medicine in Ghana (Mensah *et al.,* 2006).

There are several reports on the medicinal properties of the above-mentioned plants, and it was observed that individual work used different drying methods in order to extract the crude extracts of these plants for study. Based on this observation, this study aims at investigating the effect of different drying methods on the antimicrobial properties of these plants to ascertain the best drying method to employ.

MATERIAL AND METHODS:

Plant and Test organism source:

Plant:

Leaves of *Cassia alata, Commelina diffusa* and *Borreria verticillata* were collected at *Shodex Beautification* Landmark, Anthony, Lagos, Nigeria.

Test organisms:

Strains of bacterium and fungi were collected on prepared nutrient agar (NA) and sabouraud dextrose agar (SDA) slants respectively in McCartney bottles. These are obtained from dermatology unit of the Lagos State Teaching Hospital (LASUTH) Ikeja, Lagos State, Nigeria. These were stored in the refrigerator prior to use.

Identification of the Plant and Test organisms:

Plant:

The leaves were identified and authenticated at the Herbarium of the Department of Botany, University of Lagos.

Test Bacterium:

The sub-cultured bacterium strain was investigated using Gram staining method. A few drops of crystal violet were added onto the smear of the bacterium on a sterile glass slide for a minute, after which the excess stain was rinsed off using flowing water, Lugols iodine solution for 60 seconds. This was also rinsed off using flowing water. The smear was decolorized by adding 70% ethanol and counter stained with Safranin. A drop of oil immersion was added after which the slide was observed under the oil immersion objective lens.

Test Fungi:

A little portion of the growth colony was teased with a sterile inoculation needle and mounted in a drop of Methyl-Blue on a clean microscope slide. Covered with a cover slip, this was squashed with the butt of the inoculation needle and the excess fluid then blotted off. The preparation was examined under a light microscope with an attached camera (Motic McCamera [2000] 2.0-megapixel digital coloured camera) connected to a computer, for the microscopic photography of the Fungi. This was to observe the precise arrangement of the conidiophore and the way in which their spores are produced. The identities of these fungi were certified using their cultural, morphological characteristics as well as comparing them with confirmed representatives of different species in relevant texts such as Ellis *et al*. (2007).

Drying of the plants materials:

The collected l*eaves* were cleaned with distilled water to remove dirt particles before drying commenced. The leaves of each plant were divided into four groups. The first set of leaves were subjected to Oven dry at $40^{0}C$ for 4 days, the second set were also subjected to oven dry but at 60ºC for 4 days. The third set were sun dry for 7 days, while the last set were shade dry at room temperature ($28^{\circ}C$) for 7 days. After which each of these set of leaves were grounded into powdery form with the aid of an electric blender. The pulverized leaves were stored in air-tight containers prior extraction.

Extraction from the plant leaves:

Fifty grams of each plant leaves powder were measured and soaked in two different solvents namely; 100 ml aqueous methanol (methanol: water, 80:20 v/v)] and 100 ml distilled water for 24 hours. The extracts were filtered and the greenish-brown extracts was then concentrated using rotary-evaporator.

Antimicrobial Assay:

In-vitro antibacterial and antifungal activities were examined for each plant extracts. Antibacterial and antifungal activities of plant part extracts against the pathogenic bacterium (Gram-positive) and three pathogenic fungi were investigated using the agar disk diffusion method described by Alzoreky and Nakahara (2003). Solidified NA and SDA are used respectively for bacterial strain and fungal strains. These respective media were inoculated with 100 µl of bacterial inoculum (10^{6} CFU/ml) and fungal spore suspension (10^{5}spores/ml). These were spread over the plates using a sterile rod display to get a uniform microbial growth. After inoculum absorption by agar, four sterile filter discs (Perforated Whatman no 1, of 5 mm in diameter) already impregnated in various plant extracts 24 hours prior antimicrobial activity testing to were placed on the agar surface using forceps dipped in ethanol and flamed. The four discs were placed evenly (not closer

than 24 mm from centre to centre) on the surface of the agar plate.

Filter discs were also impregnated with chloramphenicol solution (50 mg/ml) and Griseofulvin solution (50 mg/ml) and used as a reference control. The inoculated plates were incubated at 30°C aerobically. Each plate was labelled with necessary information such as agent name, fungus name and time of inoculation. Culture plates were examined daily, zones of inhibition were read and measured using a transparent ruler according to the methods of Booth (1971).

Phytochemical screening:

Each plant extracts used in this study was subjected to preliminary phytochemical screening to quantify and qualify the presence of active compounds like alkaloids (Evans and Trease, 1989; Ogundipe and Oladipo, 2001), tannins (Houghton and Raman, 1998), flavonoids and cardiac glycosides (Software, 1993), saponins (Farnsworth, 1966), anthraquinones and phlebotomine (Van Buren and Robinson, 1981).

Statistical analysis:

The data were expressed as mean ± S.E.M. and were statistically analysed using one-way analysis of variance (ANOVA) and Duncan multiple range test (DMRT). Values were considered significant at $p < 0.05$.

RESULTS:

The results from the bacterial assay of the crude extract from the three medicinal plants (*Cassia alata*, *Borreria verticillata and Commelina diffusa*.) employed in the study showed that these plants possess antibacterial properties. Methanol proved to be a better solvent for the extraction of the bioactive ingredients from the medicinal plants. Of the four drying methods used, oven-dry at 40ºC and shade-dry methods were better drying methods compared to other drying methods (Table 1^{a}-1^{c}). The methanol extract of *C. diffusa* (table 1^{b}) oven-dry at 40ºC showed zone of inhibition of 13 mm in *Staphylococcus aureus*, which is better than that of the antibiotic (chloramphenicol) used as the positive control in the study.

The antifungal assay results reveal that the three medicinal plants possess antifungal properties which could be employed in treating various skin problems caused by fungi. Methanol extract of the leaves of *C. alata, B. verticillata* and *C. diffusa* proved to be a better solvent compare to the water extracts (Table 1^{a}-1^{c}). The results also revealed that oven-dry at 40^{0}C and shade-dry methods are better drying methods by showing clear zone of inhibition against the tested isolates compared with the other two drying methods used (oven-dry at 60^{0}C and sun-dry methods).

Results from the phytochemical screening of the plant extracts subjected to the four different drying methods revealed that drying method of plant materials has a crucial role to play in its effectiveness. Data from the quantitative and qualitative analysis of the phytochemical screening of bioactive compounds in the extracts of the three-plant subjected to different drying methods (Table 2) showed that plant extracts subjected to oven-dry at 40^{0}C and shade-dry still contained quality bioactive ingredient necessary for their antimicrobial activities.

DISCUSSION:

The results from the study show that aqueous methanolic crude extract of the leaves of *C. alata, C. diffusa* and *B. verticillata is proved to be a better extraction solvent compared to water. This finding* agrees *with the works of Mohideen et al. (2005) and* Chatha *et al*. (*2006) in which it was reported that* aqueous methanol is a good solvent for extraction from plant materials. According to Hsu *et al.* (2006), the differences in the effectiveness of the extracts of aqueous methanol and water might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants and efficiency of the extracting solvent to dissolve these endogenous compounds might very important.

From the result, the leaves dried at 40^{0}C *and those that are shade-dried show highest zone of inhibition against the isolated pathogenic fungi tested. The leaves dried at 60*^{0}C *and those dried under direct sunlight showed poor zone of inhibition against the tested fungi.* According to Orphanides *et al.* (2013), this may be as a result of the fact that high temperature might have degraded or bio transformed the bioactive ingredients in the crude extract. Hung and Duy (2012) reported that temperature above 40^{0}C will significantly reduce total free and bound phenolics, total free and bound flavonoids. This result also agreed with the work of Biesaga and Pyrzynska (2013) and Davidov-Pardo *et al*. (2011), they reported that under high temperature i.e. above the room temperature - plant phenolic is generally degraded and undergoes undesirable reactions. The outcomes of this study show that drying methods (temperature) has a crucial role to play in the effectiveness of these medicinal plants. Therefore, this suggests that the best drying method(s) to be employed for drying plant material is to either dry at 40ºC or to shade-dry.

Table 1a. Antimicrobial assay of *Cassia alata* leaves extract on bacterium and fungi isolates. (Mean inhibition zone diameter)

Isolates	60ºC		40ºC		Sun Dry		Shade Dry		Sterile Distilled water	Griseofulvin
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water		
Candida albicans	10.00 ± 0.00^{A}	00.00 ± 0.00^{A}	12.40 ± 0.55^{B}	0.00 ± 0.00^{A}	00.00 ± 0.00^{A}	11.10 ± 0.29^{AB}	11.00 ± 0.29^{AB}	12.00 ± 0.55^{B}	0.00 ± 0.00^{A}	15. 37 ± 1.05^{CD}
Trichophyton tonsarans	12.00 ± 0.55^{B}	0.00 ± 0.00^{A}	13.60 ± 1.05^{B}	10.00 ± 0.0^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.00 ± 0.55^{B}	12.00 ± 0.55^{B}	0.00 ± 0.00^{A}	13.20 ± 1.05^{B}
Trichophyton mentagrophytes var. quinckeanum	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.50 ± 0.46^{B}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.50 ± 0.55^{B}	13.50 ± 0.90^{B}	0.00 ± 0.00^{A}	13.60 ± 1.05^{B}
										Chloramphenicol
Staphilococcus aureus	12.40 ± 1.14^{BC}	0.00 ± 0.00^{A}	10.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	10.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12. 50 ± 0.55^{B}

Table 1b. Antimicrobial assay of *Commelina diffusa* leaves extract on bacterium and fungi isolates. (Mean inhibition zone diameter)

Isolates	60ºC		40ºC		Sun Dry		Shade Dry		Sterile Distilled water	Griseofulvin
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water		
Candida albicans	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.25 ± 0.41^{B}	10.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	13.60 ± 1.05^{B}	11.00 ± 0.29^{AB}	0.00 ± 0.00^{A}	15. 37 ± 1.05^{CD}
Trichophyton tonsarans	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	13.60 ± 1.05^{B}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.00 ± 0.55^{B}	11.00 ± 0.29^{AB}	0.00 ± 0.00^{A}	13.75 ± 1.16^{B}
Trichophyton mentagrophytes var. quinckeanum	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.00 ± 0.55^{B}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.87 ± 0.44^{B}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	13.62 ± 0.92^{BC}
										Chloramphenicol
Staphilococcus aureus	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	13.60 ± 1.05^{B}	0.0 ± 0.00^{A}	7.50 ± 0.90^{C}	0.00 ± 0.00^{A}	11.00 ± 0.29^{AB}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.50 ± 0.70^{AB}

Table 1c. Antimicrobial activity of *Borreria verticillata* leaves extract on bacterium and fungi isolates. (Mean inhibition zone diameter)

Isolates	60ºC		40ºC		Sun Dry		Shade Dry		Sterile Distilled water	Griseofulvin
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water		
Candida albicans	12.25 ± 0.41^{B}	10.00 ± 0.00^{A}	13.20 ± 1.05^{B}	13.00 ± 0.65^{B}	10.00 ± 0.00^{A}	0.00 ± 0.00^{A}	14.25 ± 0.70^{B}	12.00 ± 0.55^{B}	0.00 ± 0.00^{A}	15.75 ± 1.03^{C}
Trichophyton tonsarans	11.87 ± 0.51^{B}	11.00 ± 0.29^{AB}	13.12 ± 0.78^{B}	11.12 ± 0.22^{AB}	11.87 ± 0.51^{B}	10.00 ± 0.00^{A}	13.12 ± 0.78^{B}	12.00 ± 0.55^{B}	0.00 ± 0.00^{A}	13.62 ± 0.92^{BC}
Trichophyton mentagrophytes var. quinckeanum	10.00 ± 0.00^{A}	10.00 ± 0.00^{A}	12.50 ± 0.70^{AB}	10.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.25 ± 0.41^{B}	13.60 ± 1.05^{B}	0.00 ± 0.00^{A}	13.87 ± 1.28^{B}
										Chloramphenicol
taphilococcus aureus	6.50 ± 1.18^{C}	0.00 ± 0.00^{A}	11.50 ± 0.46^{B}	8.00 ± 1.19^{D}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	8.00 ± 1.19^{D}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	12.00 ± 0.55^{B}

Means (n = 3) with the same superscript letter in a column are not significantly different (p > 0.05), while means with different superscript letter are significantly different (p < 0.05) when subjected to Duncan multiple range test, DMRT (ANOVA posthoc).

Table 2. Quantitative and qualitative of phytochemical compounds present in the leave extracts of *Cassia alata*, *Borreria verticillate*, *and Commelina diffusa.*

		Phenol mg/100 g	Flavonoid mg/100 g	Saponin mg/100 g	Tannin mg/100 g	Alkaloid mg/100 g	Cardiac-glycoside mg/100 g
Plants & Drying methods	Extraction Solvent						
Cassia alata Oven dry 40^{0}C	Methanol extract	49.58	122.05	ND	66.80	27.89	40.12
Oven dry 60^{0}C		29.78	86.78	ND	60.30	ND	37.29
Shade-dry		22.71	159.11	ND	63.18	31.78	42.19
Sun-dry		ND	39.01	ND	63.48	18.36	38.96
Cassia alata Oven dry 40^{0}C	Water extract	ND	ND	14.92	ND	ND	24.55
Oven dry 60^{0}C		ND	79.63	ND	ND	ND	28.00
Shade-dry		ND	76.10	13.10	58.72	ND	30.09
Sun-dry		ND	ND	4.49	41.51	ND	36.35
Borreria verticillata Oven dry 40^{0}C	Methanol extract	27.61	125.37	15.08	60.28	25.46	37.71
Oven dry 60^{0}C		26.76	90.12	14.33	ND	ND	32.29
Shade-dry		ND	121.31	17.68	ND	ND	34.33
Sun-dry		16.06	ND	14.75	ND	ND	ND
Borreria verticillata Oven dry 40^{0}C	Water extract	23.96	ND	ND	61.91	ND	ND
Oven dry 60^{0}C		21.58	88.49	ND	44.89	ND	ND
Shade-dry		ND	107.72	ND	103.04	ND	25.41
Sun-dry		ND	96.43	ND	ND	ND	ND
Commelina diffusa Oven dry 40^{0}C	Methanol extract	14.33	100.71	ND	ND	17.04	ND
Oven dry 60^{0}C		ND	66.00	ND	ND	ND	ND
Shade-dry		19.94	124.10	ND	ND	13.60	ND
Sun-dry		ND	53.11	ND	ND	ND	ND
Commelina diffusa Oven dry 40^{0}C	Water extract	ND	ND	11.00	ND	ND	ND
Oven dry 60^{0}C		ND	ND	ND	ND	ND	ND
Shade-dry		ND	ND	12.57	62.30	ND	ND
Sun-dry		ND	ND	ND	ND	ND	ND

ND – Not detected

REFERENCES:

Alzoreky NS, Nakahara K. 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia *Int. J. Food Microbiol.,* 80(3): 223-230.

Biesaga M, Pyrzynska K. 2013. Stability of bioactive polyphenols from honey during different extraction methods. Food Chem., 136(1): 46–54.

Booths C. 1971. Introduction to general laboratory methods. In: "Methods in Microbiology. (Norris JR, Ribbons DW. Eds)". Academic Press, London, pp 47.

Burkill HM. 2000. The useful plants of West tropical Africa. Vol. 5, Families S-Z, addenda, corrigenda, cryptogamata / H.M. Burkill. Kew, England: Royal Botanic Gardens, Kew, pp. 686.

Chatha SAS, Anwar F, Manzoor M, Bajwa JR. 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasas Aceites, 57(3): 328-335.

Davidov-Pardo G, Arozarena I, Marin R. 2011. Stability of polyphenolic extracts from grape seeds after thermal treatments. Eur. Food Res. Technol., 232(2): 211–220.

Edmunds JE. 1971. Association of *Rotylenchulus reniformis* with 'Robusta' banana and *Commelina* sp. roots in the Windward Islands. Trop. Agric. (Trinidad), 48(1): 55–61.

Ellis D, Davis S, Alexiou H, Handke R, Bartley R. 2007. Descriptions of medical fungi. Second edition. Nexus Print Solutions, Underdale, South Australia, pp. 200.

Evans WC, Trease GE. 1989. Trease and Evan's Pharmacognosy. 13th Ed., London, Philadelphia: Baillière Tindall, pp. 832.

Farias RP. 2003. Biological products drying simulation in crossflow dryer. Master Thesis, Federal university of Campina Grande, Brazil, pp. 84.

Farnsworth NR. 1966. Biological and phytochemical screening of plants. J. Pharm. Sci., 55(3): 225-276.

Houghton PJ, Raman A. 1998. Laboratory handbook for fractionation of natural extracts. Chapman and Hall, London, pp. 199.

Hsu B, Coupar IM, Ken Ng. 2006. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. *Food Chem.,* 98(2): 317-328.

Hung PV, Duy TL. 2012. Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. Int. Food Res. J., 19(1): 327-332.

Mensah AY, Houghton PJ, Dickson RA, Fleischer TC, Heinrich M, Bremner P. 2006. In vitro evaluation of effects of two Ghanaian plants relevant to wound healing. *Phytother. Res.*, 20(11): 941-944.

Mohideen S, Sasikala E, Aruhaj P. 2005. Pharmacognosy of *Cassia Alata* Linn - leaves. Anc. Sci. Life, 24(4): 192-198.

Ogundipe OT, Oladipo BO. 2001. The phytochemical and antimicrobial studies of *Persea americana* Mill. (Lauraceae). Hamdard Med., XLIV: 44-50.

Orphanides A, Goulas V, Gekas V. 2013. Effect of Drying Method on the Phenolic Content and Antioxidant Capacity of Spearmint. *Czech J.* Food Sci., 31(5): 509–513.

Oztekin S, Martinov M. 2007. Medicinal and aromatic crops: Harvesting, drying and processing. Haworth food agricultural products press, New York, pp. 320.

Sadeghi-Nejad B, Deokule SS. 2009. Antidermatophytic activities of *Ixora brachiata Roxb*. *Afr.* J. Biochem. Res., 3(10): 344-348.

Sofowara A. 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, pp. 289.

Sonibare MA, Abegunde RA. 2012. Ethnobotanical study of medicinal plants used by the Laniba village people in South Western Nigeria. Afr. J. Pharm. Pharmacol., 6(24): 1726-1732.

Van Buren JP, Robinson WB. 1981. Formation of complexes between protein and tannic acids. J. Agric. Food Chem., 17(4): 7772 - 777.