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The Effect of Extraction Protocol on the Phytochemical and Antimicrobial Activities of *Lantana Camara* Leaf Extract Found within a Local Environment.

Ofeogbu Obinna,* Anyam John and Onuwa Ogah Peter

Department of Chemistry and Centre for Agrochemical Technology,
Federal University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria

ABSTRACT

In-vitro antimicrobial activities of cold-ethanolic extracts of *Lantana camara* leaves were compared to the hot-ethanolic extracts of the same plant. The organic extracts (hot and cold ethanolic extracts) were investigated against various clinical pathogens. Agar well diffusion method was used and the extracts showed significant zones of inhibition against the tested isolates. Better antimicrobial activities were obtained with the cold extract which also gave better minimum inhibitory concentration (MIC) for the selected isolates as well as minimum bactericidal concentration (MBC).The highest zone of inhibition was recorded against *Candida albicans (29mm)* using the cold extracts while moderate zone of inhibition was recorded against *Staphylococcus aureus (25mm), and Escherichia coli* (23mm). Weak antibacterial activity was recorded against *Staphylococcus aureus (17mm)* and *Streptococcus pyogenes (18mm)* using hot ethanolic extract against 25mm and 28mm respectively recorded using cold extract against the same isolate which further prove the fact that the cold extract is more effective compare to the hot extract. Also phytochemical analysis was carried out and metabolites such as alkaloids, steroid, phenol, flavonoid, phlobatannins, terpene, tanins were discovered to be present in the leave of *Lantana camara*. The results suggest that the cold-ethanolic extract has broad spectrum not only against bacterial pathogens but also fungal pathogens and can be a novel source for the development of future antimicrobial drugs from plant source which if well exploited will be safe to use compare to the synthetic antibiotic that are no longer effective against most of the pathogenic organisms.
KEYWORDS: Antibacterial activity, clinical pathogens, *Lantana camara*, zone of inhibition.

INTRODUCTION

The plant Lantana Camara L. (Fam. Verbenaceae) is common in the field of pharmacognosy for its various applications. The leaves are used in the treatment of tumors, tetanus, rheumatism, malaria and reported to possess diaphoretic, carminative, antiseptic properties (Chowdhury et. Al,2007), various parts of the plant are used in the treatment of itches, cuts, ulcers, swellings, bilicious fever, catarrh, eczema, dysentery and chest complaints of children, fistula, pustules and rheumatism(Abdulla et.al,2009). It is listed as one of the most important medicinal plants in the world (Gwanjewala et.al, 2007, Abdulla et.al, 2009).There are various species of the plant mainly differ in colour and flowers. *Lantana camara* has the potential to interrupt generation process of other species by decreasing germination, reducing early growth rates and selectively increasing mortality of other plant species (Sharma et al, 2007). However, it is listed as one of the important medicinal plants of the world (Ross 1999). *L. camara* contains lantadenes, the pentacyclic triterpenes which is reported to possess a number of useful biological activities. Several previous reports have described antifungal, (Tripathi and Shukla 2002, Kumar et al. 2006), anti proliferative (Saxena et al. 1992, Nagao et al. 2002), and antimicrobial activities of *L. camara* (Saxena et al. 1992, Juliani et al. 2002, Kasali et al. 2002, Rajakaruna et al. 2002) including termicidal activity reported recently by Verma and Verma (2006). Moreover, the hydroalcoholic extracts of the leaves have shown an effect on fertility, general reproductive performance, and teratology in rats (Mello et al. 2005). *L. camara* whole plant and plant parts viz., leaves, flowers, and essential oils have been thoroughly studied for their chemical compositions, previously and currently (Saleh 1974, Hart et al. 1976, Sharma and Sharma 1989, Siddiqui et al. 1995, Ghisalberti 2000). All these studies have revealed the presence of terpenoids, steroids, and alkaloids as major chemical constituents in *L. camara* (Saleh 1974, Hart et al. 1976, Sharma and Sharma 1989 Siddiqui et al. 1995,). However, sesquiterpenes with mainly ß-caryophyllene, zingiberene, -humulene, arcurcumene, gemacrene-D and bisabolene were reported as major leaf and flower essential oil constituents (Singh et al. 1991, 2002, Nagassoum et al. 1999, Khan et al. 2002, Andersson and Dobson 2003). Chemical composition of the whole plant and plant parts and essential oils are reported to be

***Corresponding Author:** Ofeogbu Obinna, Department of Chemistry and Centre for Agrochemical Technology, Federal University of Agriculture, P.M.B. 2373, Makurdi, Benue State, Nigeria. e-mail: obinnafor@yahoo.co.uk

influenced by genetic, geographical, and seasonal factors as well as the developmental stages of the concerned plant, its parts/tissues. Previously, Randrianalijaona et al. (2005) have reported the seasonal changes in the chemical composition of essential oils in more than seventy *L. camara* from different parts of the world. Very recently, we reported ontogenic variation in secondary metabolites such as phenolics, anthocyanins, and proanthocyanidins in *L. camara* (Bhakta and Ganjewala 2009). However, in *L. camara* very few studies have so far been focused on the influence of seasonal, genetic, ontogenic, and developmental factors of the chemical composition. Hence, this present research is aimed to study the phytochemical composition of *L. camara* leaf. In addition, their antibacterial activities will also be study both against fungal and bacterial pathogens.

MATERIAL AND METHODS

PHYTOCHEMICAL ANALYSIS

Specific qualitative tests were performed for detection of common secondary metabolites in leaf extracts such as steroids, phenol, flavonoids, terpenes, alkaloids, tannins were determined as described by Ganjewala etal 2009 and Brinda etal 1981.

SAMPLE COLLECTION AND TREATMENT

The samples of *Lantana Camara* were obtained within the premises of federal university of agriculture Makurdi. The leaves were dried for 3days under shade.

EXTRACTION:

The dried leaves were crushed using a kitchen grinder to obtain the pulverized samples which were used for extraction with ethanol (cold-batch extraction). The ethanol was recovered later by refluxing.

ANTIMICROBIAL SCREENING

The antimicrobial activities of the leave extract from the plant; *Lantana Camara* was determined using four (4) pathogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes* , *Escherichia coli*, and *Salmonella typhi)* and one fungal isolate *(Candida albicans)*. These microorganisms were obtained from the department of microbiology Ahmadu Bello University Zaria. All the microbes were maintained in slants of blood agar. They were all sub-culture into stock culture of nutrient broth. Each of the extracts weighed 1.0g were dissolve in 10ml of their solvent of extraction to obtained a concentration of 100mg/ml. this Concentration was used as the initial concentration to check the antimicrobial activities of the plant against the tested microbes. Blood agar was the medium used as the growth medium for the microbes, and the medium was prepared according to the manufacture's instruction. The medium was weighed and dissolved in measured distilled water in a conical flask, capped with cotton wool and heated to dissolve using Bunsen burner. It was then sterilized at 121^{o}C for 15minutes using autoclave. The medium was allowed to cool to 45^{o}C, and 20ml of it was poured to sterile Petri dishes and allowed to solidify. The screening of the antimicrobial activity of the extracts was carried out using the well diffusion technique. The medium was seeded with the test microbes; the inoculums of each microbe were spread evenly over the surface of the prepared plates with the aid of sterile swab. All the seeded plates were allowed to dry and a standard cork borer of 5mm in diameter was used to produce the wells on the surface of prepared plates. The extracts were introduced into the wells. The inoculated plates were all incubated at 37^{o}C for 24hours, after which the plates were observed for the zone of inhibition of the growth and the zones were measured using a transparent ruler which was recorded in millimeters.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration of the extract was determined using broth dilution method. Nutrient broth was prepared according to the manufacturer's instruction.10mls of the broth was dispersed into sets of 5 test tubes and capped. It was sterilized at 121^{o}C for 15minutes using autoclave. Mc-Far land's turbidity standard scale of 0.5 was prepared to give a turbid solution. Normal saline was used to make a turbid suspension of the microbes, the dilution of the micro-organism was done continuously in the normal saline until the turbidity matched that of Mc-Farland's scale by visual comparison, at that point the micro-organisms have a concentration of 1.5×10^{8}cfu/ml; serial dilution of the extracts at the initial concentration of 100mg/ml was constituted in the sterilized nutrient broth in the tubes. The initial concentration was obtained by dissolving 1.0g of each of the extracts in 10ml of the nutrient broth. The dilution was carried out with the aid of sterilized syringe; having obtained the different concentrations through dilution, the suspension of the test microbes was transferred into the tubes. The tubes were incubated at 37^{o}C for 24hrs, after which they were inspected for turbidity. The tubes with the lowest concentrations without the turbidity were marked and recorded as the minimum inhibitory concentration (MIC).

MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Minimum bactericidal concentration was carried out to determine whether the microbes were killed or only their growth was inhibited by the extracts. Blood agar plates were prepared according to the manufacturer's instruction, the medium was sterilized at 121^{o}C for 15minutes and poured into sterilized Petri dishes after cooled to about 45^{o}c and allowed to solidify. The contents of each of MIC serial diluted test tubes were sub-cultured by streaking on the prepared plates with the aid of sterile wire loop. All the inoculated plates were incubated at 37^{o}C for 24hrs, after which they were observed for growth. The Minimum bactericidal concentration was the plate with the lowest concentration of the extract without growth was recorded.

RESULTS

ANTIMICROBIAL ASSAY

The antimicrobial activity was carried out using hot and cold ethanol *Lantana camara* leave extract against four bacteria isolates (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli*, and *Salmonella typhi)* and one fungal isolate *(Candida albicans)* and also, the activity of the leave extracts against the tested microorganisms examined in this study were assessed by the presence or absence of inhibition zone and zone diameter, MIC and MBC values. The results of the zone of inhibition are given in table 1 and 2 while that of the MIC and MBC are given in table 3 and 4 respectively. Both the hot and cold ethanolic extract of *Lantana camara* leave exhibited a broad spectrum of antimicrobial activity against the tested microorganisms used in this study, with *Candida albicans* exhibiting the highest zone of inhibition of 29mm when cold ethanolic extract of the leave was used followed by *Streptococcus pyogenes* having a zone of inhibition of 28mm. Moderate zone of inhibition of 23mm and 25mm was recorded against *Escherichia coli, Staphylococcus aureus,* respectively. While in the case of hot ethanolic extract of *Lantana camara* leave, the highest zone of inhibition of 20mm was recorded against *Candida albicans* and *Escherichia coli.* While weak zone of inhibition of 13mm, 17mm and 18mm was s recorded against *Salmonella typhi, Staphylococcus aureus and Streptococcus pyogenes* respectively. The best MIC values that was able to inhibit the growth of the tested microorganisms was found to range between 25mg/ml and 50mg/ml because even at these low concentrations of the extract, the plant extracts of *Lantana camara* was found to be effective against most of the microorganisms that was used for this study. While in the case of MBC, it was discovered that a higher concentration of 100mg/ml is needed for both extract to be able to kill the tested microorganisms, except in the case of *Streptococcus pyogenes* and *Candida albicans* that required a lesser concentration of 50mg/ml using cold extract.

PHYTOCHEMICAL ANALYSIS

Qualitative tests carried out on *Lantana camara* leaf detected around seven common secondary metabolites in the leaf extracts such as alkaloids, phenol, terpenes, tannins, flavonoids, phlobatannins, steroids and tannins as shown in table 5 below.

TABLE: 1 ANTIMICROBIAL ACTIVITY OF HOT AND COLD EXTRACTS OF *L. Camara* LEAVE AGAINST SOME PATHOGENIC MICROORGANISMS

Test Organism	Hot Ethanolic Extract	Cold Ethanolic Extract
Streptococcus pyogenes	S	S
Staphylococcus aureus	S	S
Escherichia coli	S	S
Salmonella typhi	S	S
Candida albicans	S	S

KEY S Sensitive

TABLE: 2 ZONES OF INHIBITION OF *L. Camara* LEAVE EXTRACTS (100mg/ml) AGAINST SOME PATHOGENIC MICROORGANISMS

Test Organism	Hot Ethanolic Extract	Cold Ethanolic Extract
Streptococcus pyogenes	18	28
Staphylococcus aureus	17	25
Escherichia coli	20	23
Salmonella typhi	13	18
Candida albicans	20	29

GRAPHICAL REPRESENTATION OF ZONES OF INHIBITION OF *L. Camara* LEAVE EXTRACTS (100mg/ml) AGAINST SOME PATHOGENIC MICROORGANISMS

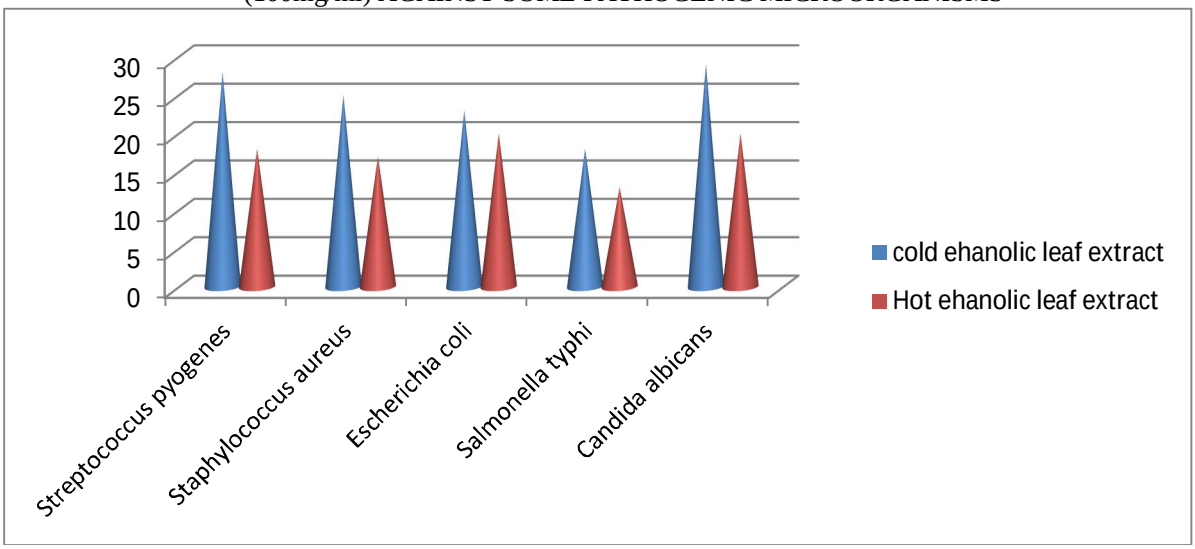

TABLE: 3 MINIMUM INHIBITION CONCENTRATION (MIC) OF *L. Camara* EXTRACTS LEAVE AGAINST SOME PATHOGENIC MICROORGANISMS

Test Organism	Leave Hot Extract					Leave Cold Extract				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Streptococcus pyogenes	-	O*	+	++	+++	-	-	O*	+	++
Staphylococcus aureus	-	O*	+	++	+++	-	O*	+	++	+++
Escherichia coli	-	O*	+	++	+++	-	O*	+	++	+++
Salmonella typhi	-	O*	+	++	+++	-	O*	+	++	+++
Candida albicans	-	O*	+	++	+++	-	-	O*	+	++

TABLE: 4 MINIMUM BACTRICIDAL CONCENTRATIONS (MBC) OF *L. Camara* EXTRACTS LEAVE AGAINST SOME PATHOGENIC MICROORGANISMS

Test Organism	Leave Hot Extract					Leave Cold Extract				
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Streptococcus pyogenes	O*	+	++	+++	++++	-	O*	+	++	+++
Staphylococcus aureus	O*	+	++	+++	++++	O*	+	++	+++	++++
Escherichia coli	O*	+	++	+++	++++	O*	+	++	+++	++++
Salmonella typhi	O*	+	++	+++	++++	O*	+	++	+++	++++
Candida albicans	O*	+	++	+++	++++	-	O*	+	++	+++

TABLE 5: RESULTS OF QUALITATIVE PHYTOCHEMICAL TEST:

Plant/Parameters	Flavonoids	Terpenes	Alkaloids	Tannins	Phlobatannins	Phenol	Steroids
Lantana camara	+	+	+	+	+	+	+

Key: + =present

DISCUSSION

The findings of this study are consisted with previous report given by Raghanvendra (2006) where the ethanol extract showed significant activities against human pathogenic bacteria viz *Klebsiella pneumoniae Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi. Similar* results were also drown by many researchers (Rabe and Vantaden, 2000; Ates and Erdogul,2003;Subramanian 2006;Manikadar etal 2006), were by majority significant activity was associated with ethanol extracts. This is due to the fact that most of the antimicrobial principles are extracted much more through alcoholic solvents. Therefore this present investigation further clearly reveals the

antibacterial nature of this plant and suggests that the plant could be exploited in the alternate antibiotic source using the cold extract over the hot extract since the plant extract was found to be more active when cold extraction method is used which may provide leads in the ongoing research of novel antibiotics. The Phytochemical compositions of *Lantana camara* leave presented in Table five are very typical of many Lantana species reported previously (Hart et al. 1976, Sharma and Sharma 1989, Siddiqui et al. 1995, Verma and Verma 2006). L. camara has been studied extensively for their antibacterial properties (Siddiqui et al. 1995, Deena and Thoppil 2000, Mello et al. 2005, Verma and Verma 2006). L. camara possess many important biological activities viz., antipyretic, antimicrobial, antimutagenic, antimicrobial, fungicidal, insecticidal, nematicidal, and others (Siddiqui et al. 1995, Deena and Thoppil 2000, Mello et al. 2005, Verma and Verma 2006). In addition, other secondary metabolites such as alkaloids, terpenoids, and phenolics could be held partially responsible for some of these biological activities (Barre et al. 1997). Therefore, antibacterial activities of *L. camara* leaf extracts reported here might be due to the presence of some of these chemical constituents in the extracts. Though, the mechanism of the action of these chemical constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. Perhaps it is one of the reasons behind differences in the antibacterial activities of the plants. Moreover, the effectiveness of the extracts varies with its concentration and the kind of bacteria used in the study. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures (Nikaido and Vaara 1985, Priya and Ganjewala 2007). vertheless, it is the ability of the active principle of the extracts that disrupt the permeability barrier of cell membrane structures and thus inhibit the bacterial growth (Nikaido and Vaara 1985, Priya and Ganjewala 2007). Finally attempts should be made to conduct in vivo studies with the extracts so as to confirm the present in vitro findings as the diameter of the zone of inhibition is not only affected by sensitivity of the microorganisms alone but also the concentration of the extract on the discs is very important and this is why in vivo findings such as toxicity of the plant to humans at different concentration is very essential before the use of the plant for treatment.

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