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Analysis of Phytochemical Composition and Bio-activity Against Clinical Pathogens of Essential Oil from Anacardium Occidentale (L.)

S. Sujatha1*, Baby Joseph2 and E.S. Antony3

1International Centre for Bioresources Management
2 Interdisciplinary Research Unit
3 Department of Microbiology, Malankara Catholic College
Mariagiri, Kaliyakkavilai, Tamil Nadu, India.

Abstract

Essential oil from nut shell part of the A. occidentale was obtained by steam distillation using Clevenger type system. The essential oil (dried aerial parts) was analysed by GC-MS techniques inorder to determine the majority compounds. As a result of GC/MS analyses, 10 compounds were identified, representing 97.95% of the total. GC/MS analyses of the oil have revealed the occurrence of Cardanol (31.1), Anacardic acid (25.3) and τ-Cadinol (21.7). For instant, of monophenolic compound (12.9%) and 1-ethyl-3-methyl-benzene, (4.5) as the major constituents of essential oil. Though, minimum percentage of peak compounds also been observed δ − Cadinene and α- Pinene showed the percentage of 3.5 and 3.7 respectively. The major components of essential oil are cardol, cardanol and Anacardic acid and the other main components identified are found in the shell of A. occidentale. When there essential oil with three different solvents such as ethanol, Methanol and Formaldehyde exposed with bacterial cells, subsequently they had an efficient antimicrobial activity against the clinical pathogens. However, among the tree solvent extract maximum bio-activity shows with ethanol than other two solvents. The present work concluded that the methanolic extract showed strong antibacterial activity against the bacteria A. chlorococcum. Though, the other hand it was proved that the methanol extracts has less activity than essential oil. The result of phytochemical screening states that in essential oil triterpenoids. Reducing sugars, alkaloids, phenolic compounds, Xanthoprotein, Tannins were moderately present. While, the methanolic extract shows that the maximum levels of compounds present such as steroid. Saponins, Phenolic compounds and tannins in essential oil of A. occidentale .

Key words: A. chlorococcum, Essential oil, Antimicrobial activity, Phyto constituents

Introduction

Higher and aromatic plants have traditionally been used in folk medicine as well as extend the shelf life of foods, inhibition against bacteria fungi and yeasts (Hullin et al., 1998). Most of their properties are due to essential oils produced by their secondary metabolism (Guillen et al., 1996). Essential oils and extracts from several plant species are able to control the microorganisms related to skin (Helander et al., 1998) and food spoilage including gram positive and Gram negative bacteria () Plant essential oil and their constituents from a variety of plants are known to possess antifungal and antibacterial activity (Torquato et al., 2004). Phenolic constituents present in essential oil are generally recognized as active antimicrobial compounds (Srinivasan et al., 2001). Generally the exact cause effect relation for made of action of phenolic compounds has not so far been determined (Valsaraj et al., 1997). However, researchers indicated that they inactive essential enzymes reacting with the cell membrane or disturbing material functionally (Gedam et al., 1972). In addition the traditional medicine approaches regarding essential oil and their antimicrobial evaluation have been recognized as valuable (Tymar et al., 2004).

Many pharmaceutical companies show interest in plant-derived drugs mainly due to the current wide spread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs which have adverse side effects

(Natarajan et al.,1999). As per the World Health Organization (WHO) report 80% of the world population, presently use herbal medicine for some aspect of primary health care (WHO, 1993).

In general A. occidental plants are used in folk medicine in the treatment of skin and venereal diseases the respiratory problems and nervous disorder (Maria and Kozukek, 2002; Watanabe et al., 2010). Evidently the phytochemical research based on ethno pharmacological information's is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Valsaraj et al., 1997). Pharmacological industries have produced a number of new antibiotics in the last three cenacles resistance to these crags by microorganisms has been increased. There over general bacteria have the genetic ability to transmit and acquire resistance as therapeutic agents (Srinivasan et al., 2001). Among the wide range of aromatic plants used in India field medicinal essential oil from A. occidentale consideation attention in the treatment of diarhorrea and vaccine for. According to our knowledge there have been ample of works conducted on the chemical compositions and pharmaceutical effects of A. occidentale (L.). Hence, the objective of the present study was to identify the volatile constituents of the essential oil from A. occidentale and also assess its invitro antimicrobial activity.

Material and Methods

Collection of sample (CNSL)

A. occidentale shell oil was purchased from the manufacturing place Near by Holy cross college, at Department of Botany Holy cross College, Nagercoil. The essential oil was gained by hydrodistillation using a Clevenger-type apparatus for 3 hrs, from the sample shell liquid. The oil obtained was dried over anhydrous sodium sulfate overnight and kept in sterile sample tubes in refrigerator. The oil yields were calculated on a dry weight basis as 0.47%.

Preparation of Extracts

A 30 g of air-dried plant crude extract of essential oil was soaked in 300 ml of organic solvents, viz., methanol, hexane and chloroform separately for 24 h in a round bottomed flask at room temperature. Extracts were filtered through the Whatman filter paper No.1. The filtrate was allowed to dry at room temperature and hexane, methanol and chloroform extracts were obtained. Condensed extracts were weighed and stored in air-tight containers at 4℃ till further investigation.

Antibacterial Activity Agar disc diffusion assay

The antimicrobial activity of essential oil and Ethanolic extract of the plant A. occidentale was investigated by disc-diffusion method on Mueller-Hinton broth. The antibacterial activity of the extracts was determined by the disc diffusion method. (Samy and Ignacimuthu, 2000). Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth to obtain a bacterial suspension of 108 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Five filter paper discs (Whatman No. 1, 6 mm diameter) were placed on the inoculated agar surface. A 20 µl of the extracts (100 mg/ml) were loaded on to the filter paper discs and were allowed to dry completely. Standard antibiotics Ampicillin (10 µg), Gentamycin (10 µg) and 20 µl of DMSO were placed as controls. Plates were incubated at 37℃ for 24 h. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicate.

Determination Of Minimum Inhibitory Concentration

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution in the Muller-Hinton broth to obtain concentrations from 100 mg/ml to 0.19 mg/ml. Standard antibiotics Ampicillin, gentamicin and DMSO were placed as controls. A 10 µl of 107 (CFU) bacterial cultures were added to the tubes and were incubated at 37℃ for 18 h. MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration.

MIC index

The MIC index (MBC/MIC) was calculated for each extract and standard control drug to determine whether an extract is bactericidal (MBC/MIC <4) or bacteriostatic (MBC/MIC >4) on growth of bacterial organisms (Samy and Ignacimuthu, 2000; Perumalsamy et al., 1999). In addition, the range of MIC index values greater than 4 and less than 32 are considered as bacteriostatic Parekh et al., (2005).

Qualitative analysis on phytochemical constituents Test for flavonoids:

A few drops of 1% NH3 solution is added to the methanolic extract of plant leaves in a test tube. A yellow coloration is observed if flavonoids compounds are present.

Test for tannins:

The 0.5 g of powdered sample of plant leaves is boiled in 20 mL of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. The 0.1% FeCl3 is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins.

Test for carbohydrates:

The 0.5 mL of powdered sample of extract, 5 mL of Benedict's reagent was added and boiled for 5 min. Formation of bluish green colour showed the presence of carbohydrate solution was boiled for few minutes. In the

presence of flavonoids, reddish pink or dirty brown colour was produced.

Test for alkaloids:

Five milliliter of the extract was added to 2 mL of HCl. To this acidic medium, 1 mL of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

Test for proteins:

To a small amount of methanolic leaves extract, 5-6 drops of Million's reagent was added. A white precipitate which turns red on heating was formed and it is indicates the presence of proteins.

Test for steroids:

One milliliter of the extracts was dissolved in 10 mL of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for terpenoids:

Five milliliter of methanolic extract is mixed with 2 mL of CHCl3 in a test tube. Three milliliter of concentrated H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Results and Discussion

Qualitative analysis carried out for methanolic extract of the leaves of A. occidentale showed the presence of five major groups of phytochemical constituents is summarized in Table 1. Phytochemical screening of the plant leaves revealed the presence of flavonoids, steroids, carbohydrates, alkaloids, tannins and terpenoids were present in A. occidentale extract. Proteins are absent in A. occidentale shell oil extract. Flavonoids are mainly found in the leaves. From the amount of precipitate formed and degree of colour change, it was deduced that the shell oil extract of A. occidentale yielded the lowest concentration of tannins. A preliminary survey of the scan of the methanolic extract of A. occidentale evidenced the presence of multiple components in the extract.

Table 1: Preliminary Phytochemical analysis of an essential oil from CNSLO with four various solvents

Name of the solvents	S	TT	R	A	PH	SA	XP	T	F	AA
Essential oil	-	+	+	-	+	-	-	+	++	+
Ethanol	-	+	+	+	+	-	+	+	_	+
Methanol	++	-	-	-	+	++	-	+	+	-
Chloroform	-	++	-	-	-	-	+	-	+	+

(-) - means Absence

+ - means moderately present

S- Steroids, TT-Triterpenoids, R-Reducing sugars, A-Alkaloids, Phenolic Compounds, SA- Saponins, XP-Xanthoprotein, Tannins, F- Flavonoids, Flavonoids and AA-Aromatic acids

The analysis of phytochemicals in the methanol leaf extracts of A. occidentale

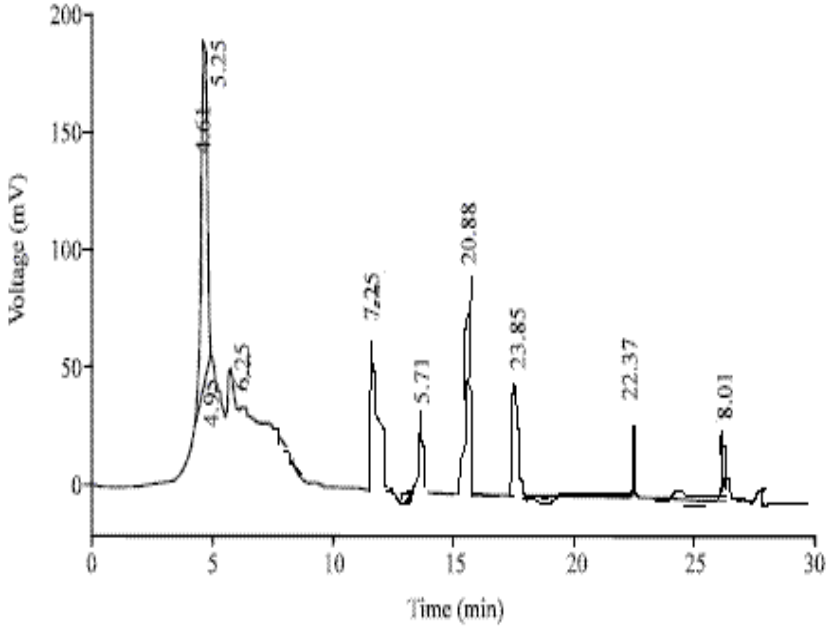

Fig.1: Gas Chromatogram of the essential oil of A. occidentale

Table-2. Phytochemical composition of the essential oil from Anacardium occidentale (L.)

Retention time	Name of the Phytochemicals	Percentage of the Composition (Peak level)
4.61	cardanol	31.1
4.95	Anacardic acid	25.3
5.25	τ-Cadinol	21.7
6.25	monophenolic compound	12.9
7.25	β-sesquiphellandrene	3.1
5.71	carvacrol	1.5
20.88	4-Terpineol	2.3
23.85	δ−Cadinene	3.5
22.37	α- Pinene	3.7
8.01	1-ethyl-3-methyl-benzene,	4.5

The essential oil was extracted by the hydrodistillation of the CNSLO and the constituents were analyzed by GC-MS. The oil yields were calculated on a dry weight basis as 0.47%. The essential oil of A. occidentale was analyzed to determine their constituents noted on the (Table 2).

As a result of GC/MS analyses, 10 compounds were identified, representing 97.95% of the total. GC/MS analyses of the oil have revealed the occurrence of Cardanol (31.1), Anacardic acid (25.3) and τ-Cadinol (21.7). For instant, of monophenolic compound (12.9%) and 1-ethyl-3-methyl-benzene, (4.5) as the major constituents of essential oil. Though, minimum percentage of peak compounds also been observed δ−Cadinene and α- Pinene showed the percentage of 3.5 and 3.7 respectively. Mono monophenolic compounds were the characteristic constituents of the oil of A. occcidentale. α- Pinene, 1-ethyl-3-methyl-benzene, sabinene, (2-methylprop-1-enyl)-cyclohexa-1, 3-diene, β-phellandrene, carvone, phellandral, 1(7), 3, 8-o-menthatriene, p-cymen-7-ol, carvacrol and γ-muurolene were found to be the minor components of essential oil in the present study (in trace) Fig.1.

Plate 1. Antibacterial activity of Essential oil from CNSLO with three various extracts of (a) Methanol, (b) Ethanol and (c) Formaldehyde

(a) **(b)** **(c)**

Table.3. Antibacterial activities of essential oil from CNSLO with three various solvents (E, M and F)

Microorganism	Inhibition zone diameter (mm)	Antimicrobial agent	
Gram positive			
	Ethanol	Methanol	Formaldehyde (300 µg /ml)
Bacillus mycoides	25 ± 0.5[a]	19 ± 0.5	34 ± 0.2*
Bacillus subtilis	26 ± 0.5**	14 ± 0.3[a]	29 ± 0.3[is]
Staphylococcus aureus	10 ± 0.3[a]	8 ± 0.3	30 ± 0.1
Gram Negative			
Agrobacterium tumefaciens	16 ± 0.4[a]	13 ± 0.5*	32 ± 0.3[a]
Azotobacter chlorococcum	28 ± 0.5**	5 ± 0.5[is]	34 ± 0.2
Enterobacter cloaceae	24 ± 0.5[*]	16 ± 0.3*	36 ± 0.5[a]
Erwinia carotovora	18 ± 0.3**	19 ± 0.5[a]	31 ± 0.5
Klebsiella pneumonea	31 ± 0.5[a]	22 ± 0.5**	10 ± 0.7[a]
Proteus sp.	–	–	11 ± 0.5[is]
Pseudomonas aeruginosa	–	–	17 ± 0.3
Pseudomonas glycinea	20 ± 0.5[a]	16 ± 0.5*	15 ± 0.7[a]
Pseudomonas fluorescens	22 ± 0.5*	12 ± 0.3[is]	11 ± 0.7*
Pseudomonas phaseolicola	23 ± 0.5*	19 ± 0.5[a*]	35 ± 0.5
Fungi			
Aspergillus niger	29± 0.5	-	20±0.5*
Fusarium oxysporum	17 ±0.5[is]	11±0.5[is]	28±0.5
Penicillium canescens	10±0.5	-	22±0.5

*- denotes statistically significant p<o.o1% level

[is-] Insignificant

- _ means no activity

Mean value ± SD, n=3 (the zone of inhibition (in millimeter) including disc of 6 mm in diameter). A solvent control (methanol) was negative.

The methanolic extract showed strong antibacterial activity against the bacteria A. occidentale, inhibition zone is 31 ± 0.5 mm against Klebsiella pneumoniae. Although both essential oil with methanol extract had similar sizes of the zone of inhibition for A. occidentale (19.0±0.5) (Table-3). In general, the essential oil showed better antimicrobial activities than the methanol extract. While, all other microorganism the methanol extract showed less activity. From these results indicates among the four solvents ethanolic extract possessed significantly peak anti potent activity against gram negative as well as gram positive bacterial organisms (Plate-1).

Discussion

Plants derived products are used as a source of medicines since long (Shilandra et al., 2010). According to World Health Organization (WHO) more than 80% of the worlds population mostly in poor and less developed countries depend on tradition plant leased medicines for their primary health ears needs (WHO, 1993). Several researchers have theorized that plant essential oils soften the walls of the bacteria then permeate them thus causing the enhanced anti-bacterial effect. In order to that Addition of at least one sesquiterpenoid to advance the antimicrobial effect of antimicrobial compounds (Paramashivappa et al., 2006). Very recently, Watanabe et al. (2010) published CNSLO having the ability to inhibit as well as the enhancing the ability to growth the ruminant gut bacteria. Though number of researchers have been identified on eleven essential oil from the plant derived products and their major constituents in the gaseous state was evaluated against Haemophilus influenza, Streptococcus pneumoniae, Streptococcus pyogenes and Staphylococcus aureus. For most essential oils examined, H. influenza was most susceptible, followed by S. pneumoniae and S. pyogenes, and subsequently S. aureus. Penicillin-susceptible and resistant S. pneumoniae were comparable in susceptibility. Escherichia coli, which was used as a control, showed least susceptibility. A minimal inhibitory dose (MID) was introduced as a measure of the vapour activity. Previously, Kone et al. (2004) viewed contradictorily additionally 14 kinds of essential oil among this lemongrass and thyme oils showed the lowest MID, mainly because of essential oils containing terpene alcohols as a major constituent. Similarly the following researchers agreed with these results such as the essential oils containing terpene ketone, ether and, in particular, hydrocarbon had high MIDs (Shilandra et al., 2010; Dahaerkan et al., 2000). The vapour activity on short exposure was comparable to that following overnight exposure, and rapid evaporation was more effective than slow evaporation of essential oils. But very ample of works has been done on this essential oil from cashew nut shell oil and its activity against pathogenic and non pathogenic microorganisms.

Previously, Samy and Ignacimuthu, (2000) viewed an antimicrobial compositions based on a combination of plant essential oils are of enhanced antimicrobial effectiveness and are prepared by adding to at least two plant essential oils a small but antimicrobial enhancing effective amount of an enhancer selected from the group consisting of polyionic organic enhancers and polyionic inorganic enhancers (Nenad et al., 2005). One preferred composition it is a mixture of plant essential oils wherein at least one of the oils is oregano oil.

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