



Phytochemical Screening and Identification of Active Ingredients in *Nerium (Nerium oleander)* L. and *Lantana (Lantana camara)*

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ABSTRACT

Powdered leaves of *Nerium oleander* L. and *Lantana camara* were screened for detection of saponins, glycosides, terpenes, flavonoids, tannins, alkaloids, cardiac glycosides, and cyanogenic glycosides. The results of the qualitative analysis and phytochemical screening profiles indicated that, most of the natural tested products are presented in *N. oleander* L. leave extracts, for *L. camara*, most of the natural products tested for are present except saponins and glycosides. The chemical compositions of the leaf ethanol extract of *N. oleander* and *L. camara* were investigated using gas chromatography –mass spectroscopy (GC-MS). Analysis of *N.oleander* leaf ethanol extract revealed the existence of twenty two components identified, of them 4-0-methylmannose, Squalene, n-hexadecanoic acid, vitamin E; 3,5-dimethoxyacetophenone; 9,12,15-octadecatrienoic acid and benzofuran-2, 3-dihydro, represent 35.92%, 14.72%, 11.10%, 7.57%, 7.13%, 6.84 %, 3.19%, respectively, the other components were ranged from 1.3 to 1.97 %. While *L. camara* major chemical component were found to be forty two of them, caryophyllene bicycle (3, 1, 0) hexane, 4-methylene-1-(1-methyl-lhumulene, bicycle (3, 1, 0) hexane-6-methanol,2-hydro, eucalyptol, caryophyllene oxide, represent 21.47%,11.68 %,10.93 %,9.75% 8.97%, 8..03%, respectively, the other components were ranged from ,1.1 to 3.53 % .

Keywords: *Nerium oleander*, *Lantana camara* , leaves, phytochemical, chemical compositions

1. Introduction

Plants and plant – derived natural products are important sources of food additives, fuel, drugs, pesticides, pigments, resins, fragrances and other significant industrial, medicinal and agricultural raw materials. Plants products can be classified as either primary or secondary metabolites. Primary metabolites are ubiquitous in nature and are needed for general growth and physiological development (Mavituna, 1992). In higher plants: such compounds are often concentrated in storage organs, e.g. sugar and starch in the roots, rhizomes, tuber, and seeds e.g. oils. Most living organisms produce the common primary metabolites and secondary metabolites are important in plant survival, because they may combat infectious disease as defensive, protective, or offensive chemicals against microorganisms and insects. They may discourage herbivores, attract pollinators also act as natural herbicides, and may represent chemical adaptations to environmental stresses. The distribution of these chemicals in the plant kingdom is usually restricted to particular pathways sometimes the same secondary metabolites may be found in untreated *taxa* e.g. nicotine related alkaloids (Mavituna, 1992).

Plants are important sources of natural products. It requires a considerable amount of time and effort to find new compounds, which have the right characteristics for use as agrochemical leads. The chances of success are greatly improved if appropriate care and consideration is given to plant screening programs, that few of these compounds may be successful as the pyrethrins (Benner, 1993). Oleander has historically been considered a poisonous Plant because some of its compounds may exhibit toxicity,

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especially to animals, when consumed in large amounts. Among these comp and are oleanderin and oleanderigenin knows as cardiac glycosides, which are known to have a narrow therapeutic index and can be toxic when ingested. Toxicity studies of animals administered oleander extract concluded that rodents and birds were observed to be relatively insensitive to oleander Cardiac glycosides (Szabuniewicz *et al.*, 1972). Other mammals such as dogs and humans are relatively sensitive to the effects of cardiac glycosides and the clinical manifestations of (glycoside intoxication) (Szabuniewicz *et al.*, 1972).

Lantana(*Lamtana camara* L) has been used to treat ailment such as common cold , chicken box, chest cold, fever, flu, sores toothaches and inflammations. The hapoglycemic and wound healing activities of *L. camara* leaf extracts were studied. for hypoglycemic activity test, leaf aqueous extracts were used on nonglycemic and alloxan-indosed hyperglycemic rates, while for wound healing activity test leaf juice and hydro alcoholic extracts (10%) were used on excised rates. The aqueous extract at 200 and 400 ml/kg significantly reduced blood glucose rates in hyperglycemic rate in 2-4 h of administration. In normal glycemic rates, 400 mg/kg extracts significantly lowered blood glucose levels, the leaf juice was more active than the leaf extract in wound healing (Dash *et al.*, 2001). The aim of this study was to conducting phytochemical chemical screening for nerium and lantana plant.

2. Materials and Methods

2.1. Sample Collection

The fully mature leaves of *N. oleander* and *L camara* were collected from Port sudan During the month of october the leave were throughly washed and air dried for 10 dayes.

2.2. Site of the experiments

The experiments were carried out in the Laboratory research of department of applied chimestry Faculty of applied Sciences, University of Red sea.

The fully mature leaves of *N. oleander* and *L. camara* were used as basic materials in this study and they were collected from Port sudan town during the month of october.

2.3. Preparation of nerium and lantana Materials

The collected samples of leaves were dried at room temperature. separated from each other and then powdered using an electric blender. The powders of both nerium and lantana leaves were kept in glass bottles for use.

2.4. Phytochemical Analysis of the leaves

Qualitative analysis was carried out for the following secondary metabolites using the follwing mentioned reageat (Table 1).

Secondary metabolites	Reagents
Saponins	Ethanol (50%)
Glycosides	Glacial acetic acid, H ₂ SO ₄ , FeCl ₃
Flavonoids	HCl , NaOH
Tannins	Et.OH, Fe Cl ₃ (5%, w/v in methanol)
Sterols and triterpenes	petroleum ether, chloroform, acetic anhydride +H ₂ SO ₄
Alkaloids	Mayer's
Coumarins	Sodium hydroxide solution
Cardiac glycosides	Liebermann-Burchard ,BaljetKeller-Killiani's
10- Anthraquinones glycosides	FeCl ₃ , H ₂ SO ₄ , chloroform and Ammonium hydroxide

3. Results

3.1. Phytochemical screening

The results of the qualitative phytochemical analysis (Tables 2 and 3), indicated that most of the tested compounds were present in *N. oleander* plant. For *L camara*, most of the tested compounds of natural products are present, except saponins and glycosides. They are grouped as saponins, glycosides, flavonoids, tannins, alkaloids, coumarins, cardiac glycosides, and sterols Triterpenes (Table, 2)

Table 2: Results of the simple phytochemical tests of the secondary metabolites of *lantana* and *nerium* leaves (+ detected; - not detected).

Secondary Metabolites	Leaves		Reagents	Observation/ Result
	Lantana	Nerium		
Saponins	-	+	Ethanol (50%)	Voluminous froth (honey comb) was developed
Glycosides	-	+	Glacial acetic acid, H ₂ SO ₄ , Fe Cl ₃	Reddish brown layer at the interface was formed and the upper layer gradually acquired a bluish-green colour, which darkened on standing.
Flavonoids	+	+	HCl, NaOH	A yellow color was formed
Tannins	+	+	Et. OH Fe Cl ₃ (5%, w/v in methanol)	Appearance of green colour, which changes to a bluish black colour or precipitate
Sterols and triterpenes	+	+	petroleum ether, chloroform, acetic anhydride +H ₂ SO ₄	Formation of a reddish violet ring at the junction of the two layers
Alkaloids	+	+	Mayer's	A precipitate was formed (turbidity)
Coumarins	+	+	Sodium hydroxide solution	Appearance of blue or green colour under UV
Cardiac glycosides	-	+	Liebermann-Burchard Baljet Keller-Killiani's	A change in colour to green or blue. A colour change to red or orange ring around the spots. A brown ring is formed in between the two layers.
Anthraquinones glycosides	-	+	FeCl ₃ , H ₂ SO ₄ , chloroform and Ammonium hydroxide	A change in the colour of the alkaline layer from a pink to red colour

3.2. Chemical composition of ethanol extract from nerium

The components of ethanol extract of *Nerium* identified by GC-MS are illustrated in Table (3) among twenty two components identified, 4-o-methylmannose, squalene, n-hexadecanoic acid, vitamin E, 3,5-Dimethoxyacetophenone, 9,12,15-octadecatrienoic acid, Benzofuran, 2, 3, dihydro, represent 35.92%, 14.72%, 11.10%, 7, 57%, 7.13%, 6.84 %, 3.19% respectively, of the total oil extracted by soxhelt apparatus, the other components were in the range of ,13- 1.97 %.

Table 3: Chemical composition of nerium ethanolic extract.

No.	Chemical name
1	Cyclopropane nonyl
2	1,4,3,6-dianhydro-alpha-d-Glucopyranos
3	Benzofuran,2,3-dihydro
4	Isosorbide
5	Methoxy -4- vinylphenol
6	3,5-dimethoxyacetophenone
7	3,5-dimethoxyacetophenone
8	1-pentadecene
9	4-0- methylmannose
10	Phytol, acetate
11	Hexadecanoic acid,methyl ester
12	n-hexadecanoic acid
13	9,12- octadecadienoic acid(z,z)-,methyl ester
14	9-octadecenoic acid (z)-,methyl ester
15	Phytol
16	Linoleic acid ethyl ester
17	Oleic acid
18	9,12,15-octadecatrienoic acid,(z,z,z)
19	Octadecanoic acid
20	Squalene
21	1,6,10,14,18,22-tetracosahexaen-3-ol,2,6,1
22	Vitamin E

3.3. Chemical composition of ethanol extract of lantana

The components of ethanol extract of lantana identified by GC-MS are illustrated in Table (3) among forty two components identified, caryophyllene, bicycle (3, 1, 0) hexane, 4-methylene-1-(1-methyl), lhumulene, bicycle (3, 1, 0) hexane-6-methanol, 2 hydro, eucalyptol, caryophyllene oxide, represent 21.47%, 11.68%, 10.93%, 9.75% 8.97%, 8..03%, respectively , of the total oil extracted by soxhelat apparatus, the other components were in the range of ,1.1- 3.53 % (Table ,3).

Table 3: Chemical composition of Lantana ethanolic extract identified by GC-MS.

No.	Chemical name
1	Bicyclo(3,1,0)hex-2-ene,2-methyl-5-(1-methyl)
2	Alpha-pinene
3	Camphene
4	Bicyclo(3,1,0)hexane,4-methylene-1-(methyl)
5	Beta-pinene
6	Beta-myrene
7	carene
8	Benzene,1-methyl-3-(1-methylethyl
9	d-limonene
10	eucalyptol
11	Trans-beta,-ocimene
12	1,3,6,-Octatriene,3,7-dimethyl,(z)
13	Gamma,-Terpinene
14	2-Carene
15	Butanoic acid,2-methyl-,2-methylbutyles
16	Camphor
17	Acetic acid,octyl ester
18	carvone
19	Cyclohexane,1-ethenyl-1-methyl-2-(1-methylethylidene)
20	Copaene
21	6-epi-shyobunol
22	Beta-copaene
23	Cyclohexane,1-ethyl-1-methyl-2-4-bis(1-methylethenyl)
24	Caryophyllene
25	Beta-copaene
26	Gamma-Elemene
27	Humulene
28	Alloaromadendrene
29	Gamma-Muurolene
30	1,6-Cyclodecadiene,1-methyl-5-methylene
31	Trans-beta-Ionone
32	Davana ether
33	1,5-Cyclodecadiene,1,5-dimethyl-8-(1-methylidene)
34	Naphthalene,1,2,3,5,6,8,a-hexahydro-4,7-dimethyl-1-(1-methylethyl)
35	Spiro[2.4]heptanes-5-methanol,5-hydroxy
36	5-Hepten-3-one,2-(5-ethenyltetrahydro-5-methyl-2-furanyl)-6-methyl
37	Cyclohexane,1,4-dimethyl-2-(2-methylpropyl
38	Caryophyllene oxide
39	1,2-Dihydropyridine,1-(1-oxobutyl)
40	3-Cyclohexen-1-carboxaldehyde,3,4-dimethyl
41	Bicyclo[3.1.0]hexane-6-methanol
42	Nerolidyl acetate

4. Discussion

The results of the qualitative analysis and phytochemical screening profiles indicated that, most of the tested natural products were present in the plant material except saponins and glycosides for *L. camara*. These were saponins, glycosides, flavonoids, tannins, alkaloids, coumarins, cardiac glycosides, anthraquinones and terpenes, from different literature it was reported that antifungal activities of leaf extracts might be due to secondary metaboltes such as alkaloids, phenolic flavonoids, triterpene

glycosides, sterols, and saponins compounds these are in line with the findings of Karthikeyan *et al.*, (2009) and Lozoya and Lozoya (1989). The letters stated that the toxic properties of some plants are mainly due to the presence of various blends of different composition, which occur as secondary metabolites, on the other hand, Gordon and David (2001) reported that plant-based natural constituents can be derived from any part of the plant, like bark, leaves, flowers, roots, fruits, seedsetc.. As mentioned earlier in the literature review, secondary metabolites are involved in the process of co-evolution between plants and other organisms (Berenbaum, 1995), or antagonistic fungi (Vander *et al.*, 2000), dissuasive substances to resist insects (Wierenga and Hollingworth, 1992), pathogenic microorganisms (Berenbaum, 1995) and competitive plants.

The GC-MS analysis of crude ethanol extract of *N. oleander*, showed presence twenty two different compounds in it on the basis of the peak area percentage compounds like, 4-0-Methylmannose, Squalene, n- Hexadecanoic acid, Vitamin E, 3, 5-Dimethoxy-acetophenone, 9, 12, 15,- octadecatrienoic acid, Benzofuran, 2, 3, dihydro, represent 35.92%, 14.72%, 11.10%, 7, 57%, 7.13%, 6.84 %, 3.19 % respectively , of the total oil extracted by soxhelt apparatus, the other components were in the range of ,13- 1.97 % can be deliberate as the major compounds present in crude ethanol extract two new compounds heptacosane -3-ethyl -5- hydroxyhexanoate and 4-oxooctyl -2-hydroxyundecanoate were isolated by Sharma *et al.*, (2012) from *Nearium oleander*. The composition of the constituents of *N. oleander* from this study was similar to that obtained by other researcher, squalene is triterpene antibacterial, lipoxygenase inhibitor, pesticide Yamuna *et al.*, (2017), 9, 12, 15 octadecatrienoic acid nematocidal, fungi inhibitor, antimicrobial, n-Hexadecanoic acid (palmitic acid) is an ester compound of fatty acid and possess various activities such as hemolytic, nematocidal, pesticide and 5- α - reductase inhibitor, Soosairaj *et al.*, (2016). 9, 12-octadecadienoic acid {linoleic acid} anti-inflammatory, nematocidal, pesticide, 5- α - reductase inhibitor (Pooja *et al.*, 2017).

The GC-MS analysis of crude ethanol extract of *Lantana camara*, showed presence forty two different compounds in it on the basis of the peak area percentage compounds like caryophyllene bicyclic (3,1,,0) hexane, 4-methylene-1- (1-methyl, humulene, bicyclic (3,1,0) hexane-6-methanol, 2-hydro, eucalyptol, caryophyllene oxide, represent 21.47%, 11.68%, 10.93%, 9.75% 8.97%, 8.03%, respectively, of the total extracted by soxhelt apparatus, the other components were in the range of 1.1- 3.53. Pooja *et al.*, 2017 reported that D-limonene is high antifungal activity among twenty two naturally occurring monoterpenoids screened for activity against *Botrytis cinerea* and *Monilia fructicola* causing grey mould and brown rot disease, the phenolic monoterpenoids carvone was the most effective in inhibiting spore germination and mycelia growth of the pathogen. Narayana samy (2006), Benzoic acid derivatives have been shown to be the best inhibitors of some of the major postharvest pathogen, such as *Alternaria* spp, *Fusarium oxysporum* and *F. solani*, Narayana samy (2006). This is the first time *Davan ether* has been reported from the species of *L. camara*, all the species may present an alternative source for natural davanone. Caryophyllene oxides had been reported to possess antimicrobial and growth inhibitor (Yang and Michel, 1999). The composition of the constituents of *lantana camara* from this study different from that obtained by Kasali (2004) he was reported major component of the essential oil of *Lantana camara* leave in his study to be Sabinene (19.6%) and α - humulene (5.8%), 1,8-cineole (12.6%) , β - caryophyllene (12.7%), on the other hand Oyediji *et al.*, (2003) reported 56 components with β -caryophyllene (24.6%), α - humulene (19.5 %) Sabinene (8.8%) germaacrene D (5.7%) and Cubebol (5.7%) as major identified constituents, Oyediji *et al.*, (2003) the component of their report also markedly differ from what was obtained in this study, suggesting the existence of different chemo type rich in caryophyllene (21.47 %) and caryophyllene had been reported to possess antimicrobial and growth , Oyediji *et al.*, (2003) The ethanol extract in this study was also different from that reported by Seth *et al.*, from Uttarakhand, India, Seth *et al.*, (2012). In Brazil, commercial *Lantana oil* rich bisabolne derivatives (about 56%) had been reported, Weyerstahl *et al.*, (1999). However, Da Silva *et al.*, (1999) found different composition from three different samples from different locations in Brazil. The chemical composition of the ethanol extract of *Lantana camara* described in this study agree quite well with those previously reported in the literature, however, there were differences in relative quantities of compounds among the main compound caryophyllene, Zoubiri and Baaliouamer (2011) have been reported from essential oils of *Lantana camara* leave in other studies).

5. Conclusion and Recommendations

Results of the qualitative analysis and phytochemical screening profiles in *L. camara* indicated that, most of the tested natural products were present in the plant material except saponins and glycosides in *L. camara* plant. Twenty two and forty two chemical constituents have been isolated from ethanolic extract of *L. camara* and N.olendear respectively using Gas chromatography Mass spectrometry (GC-MS). Additional research is required before we can consider industrial application of these plant extracts, including the evaluation of their potentiality in human health and environmental risks associated with their application. Further studies should be also undertaken to elucidate the exact mechanism of action by which extracts exert their mode of actions.

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