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 %,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,
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(\textit{Lantana 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 would healing activities of \textit{L. camara} leaf extracts were studied. for hypoglycemic activity test, leaf aqueous extracts were used on nonglycamic 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 hyperglycamic rate in 2-4 h of administration. In normal glycamic rates, 400 mg/kg extracts significantly lowered blood glucose levels, the leaf juice was more active than the leaf extract in woud healing (Dash et al., 2001). The aim of this study was to counducting phytochemical chemical screening for nerium and lantana plant.

2. Materials and Methods

2.1. Sample Collection

The fully mature leaves of \textit{N. oleander} and \textit{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 chimistry Faculty of applied Sciences, University of Red sea.

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 following mentioned reageat ( Table 1).

| Secondary metabolites         | Reagents                                      |
|-------------------------------|-----------------------------------------------|
| Saponins                      | Ethanol (50%)                                 |
| Glycosides                    | Glacial acetic acid, H\_2SO\_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\_2SO\_4 |
| Alkaloids                     | Mayer’s                                       |
| Coumarins                     | Sodium hydroxide solution                     |
| Cardiac glycosides            | Liebermann-Burchard ,BaljetKeller-Killiani's   |
| 10- Anthraquinones glycosides | FeCl\_3 , H\_2SO\_4 chloroform and Ammonium hydroxide |

3. Results

3.1. Phytochemal screening

The results of the qualitative phytochemical analysis (Tables 2 and 3), indicated that most of the tested compounds were present in \textit{N. oleander} plant. For \textit{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 |
|-----------------------|--------|----------|---------------------|
| Saponins              | *Lantana* | *Nerium* | Ethanol (50%)        |
| Glucosides            | -      | +        | Glacial acetic acid, H₂SO₄, FeCl₃ |
| Flavonoids            | +      | +        | HCl, NaOH            |
| Tannins               | +      | +        | Et. OH FeCl₃ (5%, w/v in methanol) |
| Sterols and triterpenes| +     | +        | Petroleum ether, chloroform, acetic anhydride +H₂SO₄ |
| Alkaloids             | +      | +        | Mayer’s             |
| Coumarins             | +      | +        | Sodium hydroxide solution |
| Cardiac glycosides    | -      | +        | Liebermann-Burchard Baljet Keller-Killian’s |
| Anthraquinones glycosides | -   | +        | FeCl₃, H₂SO₄, chloroform and Ammonium hydroxide |

Voluminous froth (honey comb) was developed
Reddish brown layer at the interface was formed and the upper layer gradually acquired a bluish-green colour, which darkened on standing.
Appearance of green colour, which changes to a bluish black colour or precipitate.
Formation of a reddish violet ring at the junction of the two layers.
Appearance of blue or green colour under UV.
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.
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, Benzosfuran, 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-o- methylmannose |
| 10  | Phytol, acetate |
| 11  | Hexadecanoic acid,methyl ester |
| 12  | n-hexadecanoic acid |
| 13  | 9,12-octadecadienoic acid(z,z),methyl ester |
| 14  | 9-octadeconoic 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, llhumulene, 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-myreene                                           |
| 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-methylethyl)     |
| 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-lonone                                      |
| 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-ethylenetrahydro-5-methyl-2-furanyl)-6-methyl |
| 37  | Cyclohexane,1,4-dimethyl-2-(2-methylpropyl)             |
| 38  | Caryophyllene oxide                                    |
| 39  | 1,2-Dihydroxypridine,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 metabolites 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, seeds ……etc.. As mentioned earlier in the literature review, secondary metabolites are involved in the process of co-evolution between plants and other organisms (Berembaum, 1995), or antagonistic fungi (Vander et al., 2000), dissuasive substances to resist insects (Wierenga and Hollingworth, 1992), pathogenic microorganisms (Berembaum, 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-O-Methylmannose, Squalene, n- Hexadecanoic acid, Vitamin E, 3, 5-Dimethoxy-acetophenone, 9, 12, 15-octadecatrienionicacid, Benzofuran, 2, 3, dihydro, represent 35.92%, 14.72%, 11.10%, 7, 5%, 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-o xooyctyl -2-hydroxyundecanoate were isolated from Sharma et al., (2012) from Nearthium oleander. The composition of the constituents of N. oleander from this study was similar to that obtained by other researcher, squalene is triterpenic antibacterial, lipoxygenase inhibitor, pesticide Yamuna et al., (2017), 9, 12, 15 octadecatrienionic acid nematicide, fungi inhibitor, antimicrobial, n-Hexadecanoic acid (palmitic acid) is an ester compound of fatty acid and possess various activities such as hemolytic, nematicide, pesticide and 5-alpha – reductase inhibitor, Soosairaj et al., (2016). 9, 12-octadecadienionic acid (linoleic acid) anti inflammatory, nematicide, pesticide, 5- alpha-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 caryophylle bicycle (3,1,0) hexane, 4-methylene-1-(1-methy, llhumulene, 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 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 carve 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 ssp, 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- caryophylle (12.7%), on the other hand Oyedeji et al., (2003) reported 56 components with β-caryophyllene (24.6%), α - humulene (19.5%) Sabinene (8.8%) germacrene D (5.7%) and Cubebol (5.7%) as major identified constituents, Oyedeji et al., (2003) the component of their report also markedly differ from what was obtained in this study, suggesting the existence of different chemotype rich in caryophyllene (21.47 %) and caryophyllene had been reported to possess antimicrobial and growth , Oyedeji 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 bisabolone derivatives (about 56%) had been reported, Weyerstahl et al., (1999). However, Da Silva et al., (1999) found different composition from three different sample s 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. oleander* 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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