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Bioactive constituents in ethanolic extract leaves and fruit juice of *Morinda citrifolia*

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ABSTRACT

Plants and fruits are considered as one of the main sources of biologically active compounds, and several studies reported that medicinal plants are used by 80% of the people living in rural areas as primary healthcare system. Morinda citrifolia has been heavily promoted for a wide range of uses, including antibacterial, antiinflamatory, analgesic, antioxidant, and antitumor effects. The objective was to identify chemical constituents in leaves extract and fruit juice of Morinda citrifolia by GC-MS analysis. The bioactive constituents in ethanolic extract leave and fruit juice of Morinda citrifolia were investigated using Gas Chromatography-Mass Spectrometry GC-MS analysis. Bioactive constituents studies of ethanolic extract leaves and fruit juice of M. citrifolia by GC-MS analysis clearly showed the presence of eighteen and fifteen compounds, respectively. Among the identified compounds of medical importance were detected (octanoic acid, cyclopropyl, hexanoic acid, n-decanoic acid, allantoin, sorbitol, mannitol, glycerin and gamma-tocopherol).

Key words: Rubiaceae, *Morinda citrifolia*, noni, chemical constituents, extract, GC-MS.

INTRODUCTION

Plant use in treating diseases is as old as civilization and traditional medicines are still a major part of habitual treatments of different diseases. In recent times and due to historical and cultural reasons, folk medicine has taken an important place especially in developing countries where

limited health services are available. However, the absence of scientific evaluation of medicinal plants to validate their use may cause serious adverse effects [1-3].

Plants and fruits are considered as one of the main sources of biologically active compounds, and several studies reported that medicinal plants are used by 80% of the people living in rural areas as primary healthcare system. It has been estimated that about 50% of the prescription products in USA and Europe are originating from natural products including plants or their derivates [4-6].

Out of the 250,000-500,000 plant species on earth, only 1-10% has been studied chemically and pharmacological for their potential medicinal value [7].

In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous. Examples of these compounds include phenols, phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates. Such plant derived natural products are the main focus of many scientists to develop new medication for different diseases, like cancer and microbial infection [8-10].

Morinda citrifolia Linn (Rubiaceae) is one of the most important traditional Polynesian medicinal plants. The leaves are 8-10 inches long oval shaped, dark green and shiny, with deep veins. Remedies from isolated Polynesian cultures, such as that of Rotuma, illustrate traditional indications that focus upon leaves, roots, bark, and green fruit, primarily for topical aliments [11]. *Morinda citrifolia* has been heavily promoted for a wide range of uses, including antibacterial, antiinflamatory, analgesic, antioxidant, and antitumor effects [12-14]. In this study, the purpose was to identify chemical constituents in leaves extract and fruit juice of *Morinda citrifolia* by GC-MS analysis.

MATERIALS AND METHODS

Biological material

Morinda citrifolia leaves and fruit were collected from Veracruz-Mexico (Figure 1) and identified by Mrs. Allen Coombes, plant taxonomic and curator, Benemérita Universidad Autónoma de Puebla-Herbarium. The voucher specimen was deposited at the herbarium (Specimen number: 20253).

Preparation of leaves extract

Morinda citrifolia leaves (100 g) were washed with water, air dried and powered in an electric blender. Then 90 g of the powered was suspended in 100 ml of ethanol for 20 h at room temperature. The mixture was filtered by paper Whatman No. 1. The filtrate was placed in an oven to dry at 40^{o} C, the clear residue obtained was used for the study.

Preparation of fruit juice

The mature fruit (1.5 kg) were washed with water and cut into small pieces and liquidized using an electrical blender, juice obtained (about 500 ml/1.5 kg of fruit, 100% *Morinda citrifolia* fruit juice).

Gas chromatography-mass spectrometry

All regents were analytical grade or higher. Solvents were of HPLC grade and obtained from Merck (Darmstadt, Germany). GC-MS analysis was carried out on Agilent Technologies 5973 Network system (CA, USA) equipped with an HP6890 DB-5 column (30 m length x 0.25mm id. 0.25 µm film thickness). Oven temperature was set at initial temperature of 56^{o} C/1 min, increased to 194^{o} C with 12^{o} C/1 min increments, followed by a ramp of 10^{o} C/1 min to 280^{o} C/15 min. The inlet temperature was 250^{o} C and the transfer line temperature was 280^{o} C. The carrier gas was helium 1.0 ml/1 min, the comparison of the spectral data was with the NIST02 GC-MS library.

RESULTS AND DISCUSSION

Interpretation on mass spectrum GC-MS was conducted using the database NIST, having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum components stored in the NIST library.

Bioactive constituents studies of ethanolic extract leaves and fruit juice of *Morinda citrifolia* by GC-MS analysis clearly showed the presence of eighteen and fifteen compounds, respectively. The active principles with retention time (RT) and concentration (Area %) are presented in table 1 and 2. Among the identified compounds of medical importance were detected (octanoic acid, cyclopropyl, hexanoic acid, n-decanoic acid, allantoin, sorbitol, mannitol, glycerin and gamma-tocopherol).

Based on the results from trials 1 and 2, intestinal samples from chicks with 0.7% and 1.4% octanoic acid and the positive control (0% octanoic acid) were collected for enteric morphometric analysis in trial 3 on day 15 of the experiment. The results of this study demonstrate that select doses of octanoic acid, when fed for only 3 days, can consistently reduce enteric *Campylobacter* populations in young chickens already colonized with the bacterium [15]. Isaacs *et al*., (1995) observed that octanoic acid is effective in killing human infant pathogens such as herpes simplex virus (HSV-1), respiratory syncytial virus (RSV), *Haemophilus influenzae*, and group B streptococci [16].

Cyclopropil and cyclobutil are expected to be non-toxic to humans at the concentration required to eliminate *E. histolytica* trophozoites. Similarities between *E. histolytica* alcohol dehydrogenase 2 (EhADH2) and the *G. lamblia* AdhE enzyme indicate that cyclopropyl and cyclobutyl could be effective drugs for treatment of both amoebiasis and giardiasis [17].

The use of fatty acids (hexanoic acid and n-decanoic acid) is another possible method of controlling *Ascaris*. The toxicity of hexanoic acid to bacteria, e.g., *Escherichia coli*, *Streptococcus*, *Staphylococcus*, fungi, and insects has been reported [18-20]. Allantoin is a natural healing; it is characterized by accelerating the process that causes the skin to remove

dead cells and replacing aging or other new and healthy. The human body metabolizes sorbitol slowly. Sorbitol has important advantages over fructose, lower calorific value and is not a sugar. Mannitol acts on the glomerulus of the nephron, faciliting water infiltration and increasing its excretion. Glycerin exhibit antiseptic and laxative properties, and gamma-tocopherol exhibit the main function of the different variants of the antioxidant tocopherol, vitamin E itself, so must eat foods that contain, or artificial supplements [13].

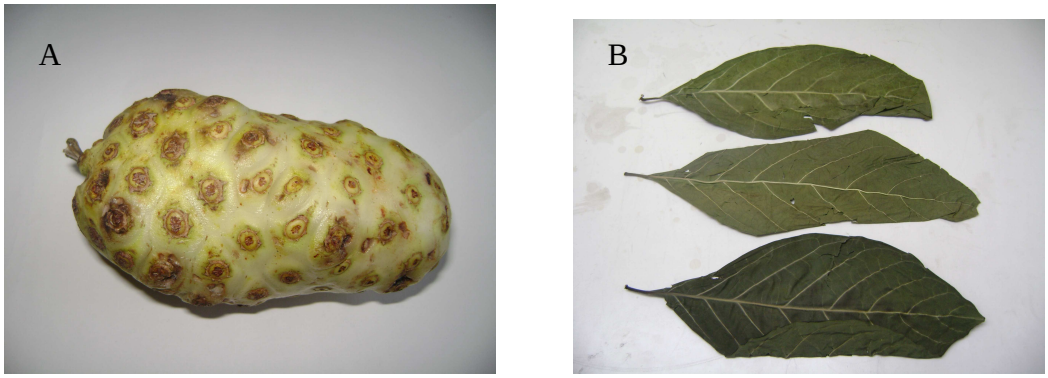

Figure 1. Biological material used for analysis, (A) fruit and (B) leaves of *M. citrifolia*.

Table 1. Components identified in extract leaves (GC-MS)

Pk #	RT	Area %	Library/ID	Ref #	CAS #	Qual
1	4.42	1.19	Glycerin	2373	000056-81-5	72
2	7.63	0.19	D-Arabinitol	24429	000488-82-4	50
3	9.98	0.32	Pentadecane	64575	000629-62-9	96
4	10.99	4.57	Hexadecane	73964	000544-76-3	97
5	11.59	1.09	D-Arabinitol	24429	000488-82-4	46
6	11.69	1.55	Sorbitol	44278	000050-70-4	62
7	11.96	1.98	Heptadecane	82607	000629-78-7	97
8	12.71	0.79	Tetradecanoic acid	75072	000544-63-8	95
9	13.07	0.79	Octadecane	91037	000593-45-3	93
10	14.18	0.89	Heptacosane	151556	000593-49-7	91
11	14.98	2.68	n-Hexadecanoic acid	92228	000057-10-3	98
12	16.47	4.88	Phytol	115539	000150-86-7	90
13	17.18	0.15	Octadecanoic acid	124072	000111-61-5	93
14	18.72	0.09	Eicosanoic acid	124052	000506-30-9	98
15	25.21	0.06	Gamma-tocopherol	159683	007616-22-0	93
16	26.45	0.21	Vitamin E	161945	000059-02-9	99
17	28.19	0.28	Campesterol	156588	000474-62-4	99
18	28.83	0.27	Stigmasterol	158840	000083-48-7	99

Three cases of particular interest are described, which clearly demonstrate the three essential properties of *Morinda citrifolia*. 1) The drugs hypotensive action is probably similar to that of *Rauwolfia serpentine*, but different from usual hypotensor, including the ganglioplegics. Its effects are slow to manifest themselves and probably are obtained indirectly through slow and progressive improvement in the state of the arteriovenous system and the supporting connective tissue. 2) *Morinda citrifolia* calms the sympathetic nervous system are the first to be benefited by the drug. 3) The anticongestive property of *Morinda citrifolia* was shown in its beneficial action on hemorrhoids, nasal congestion, and cerebral hemorrhage. The drug has other good qualities

that will doubles render it of value in the treatment of other conditions besides hypertension [21]. *Morinda citrifolia* prevents cancer at the initiation stage of c arcinogenesis, antioxidants activities of fruit juice showed a dose dependence *in vitro* against lipid peroxides and superoxides anion radical. The blockage of chemical carcinogen-induced DNA adducts and the strong antioxidant activity of fruit juice may contribute to the cancer preventive activity of *Morinda citrifolia* at the initiation stages of chemical carcinogenesis [22,23], also prevents ischemic neuronal damage through suppression of the development of post post-ischemic glucose intolerance [24] and immunostimulant activity on T and B lymphocytes [25]. In conclusion, both leave extract and fruit juice of *Morinda citrifolia* contain chemical compounds of importance in health, and the potential isolation and use of new bioactive products from plant origins is still very productive playground for the development of new drugs to improve health care in certain medical fields.

Table 2. Components identified in fruit juice (GC-MS)

Pk #	RT	Area %	Library/ID	Ref #	CAS #	Qual
1	4.56	3.53	Hexanoic acid	7851	000142-62-1	90
2	5.82	1.40	Cyclopropyl carbinol	674	002516-33-8	39
3	6.80	6.82	Octanoic acid	19985	000124-07-2	90
4	7.44	3.25	2-Furancarboxaldehyde	10771	000067-47-0	76
5	8.83	0.14	n-Decanoic acid	37193	000334-48-5	91
6	10.01	0.32	d-Mannitol	32132	007726-97-8	40
7	10.37	0.21	2-Carbamyl	114507	121358-21-2	47
8	11.84	0.34	Vitamin d3	152557	001406-16-2	30
9	12.94	0.04	Allantoin	28385	000097-59-6	25
10	14.61	0.07	Pentadecanoic acid	100724	005129-60-2	95
11	15.15	1.24	n-Hexadecanoic acid	92228	000057-10-3	97
12	17.01	14.11	9, 12-Octadecanoic acid	106289	000060-33-3	96
13	19.56	1.44	Cyclododecyne	31957	001129-90-4	93
14	27.51	28.79	1, 5-Cyclodecadine	15192	001124-78-3	86
15	30.41	6.75	5. alpha-Androstan	122230	000564-29-4	46

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