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GC-MS ANALYSIS OF METHANOLIC STEM BARK EXTRACT OF *Indigofera zollingeriana*

J. O. ALAGBE[1*], M. D. SHITTU[2] AND F. T. USHIE[3]

[1]Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India.
[2]Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.
[3]Department of Agricultural Technology, Federal College of Forestry Mechanization, Afaka Kaduna State, Nigeria.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to examine the bioactive chemicals in methanolic stem bark extract of *Indigofera zollingeriana* using Gas Chromatography – Mass Spectrum (GC-MS). The result revealed the presence of 19 compounds with peak percentage in chromatogram. The major phytoconstituent in the exatract are: Indigoferabietone (21.67 %), Indigo, 2, 2-bisindole (14.35 %) and Indigoidin (12.49 %) with retention time of 17.38 min, 20.57 min and 10.33 min respectively. It was concluded that the presence of various bioactive chemicals or phytochemicals in *Indigofera zollingeriana* stem bark justifies that it can be used for the treatment of many diseases in animals and could be used as an alternative to antibiotics to promote food safety.

Keywords: GC-MS analysis; *Indigofera zollingeriana* stem bark; methanol; phytochemicals.

1. INTRODUCTION

One of the numerous important gifts of nature are plants; they contain bioactive chemicals that have medicinal properties and have been used traditionally to treat different ailments because physiological action in the body of animals [1]. About 80 % of the world population relies on medicinal plants because of the assumed minimal side effects, effectiveness and low cost proceurement (Kumara, 2001) [2]. Plants have been effectively used to promote food safety and safeguarding the health of human being and animals.

The genus *indigofera* belongs to the family fabaceae which is ranked the third largest family of the blossoming plants after Orchidnaceae and Asteraceae with approximately 650 genera and 18,000 different species [3]. The family Fabaceae is divided into three sub-families (*Caesalpiniodeae, Mimosoideae, Fabiodeae*) characterized by the pod type of fruit developing from a single carpal with marginal placentaion [3].

Scientific studies have shown that *Indigofera spp* exhibit antimicrobial, hepatoprotective, anti-inflammatory, antioxidant, hypolipidemic, antimalarial, antidiabetic, antipasmodic and lipoxygenase inhibitory activities [4,5]. The root and stem bark have been traditionally used for the treatment of cough, hepatitis, tooth ache, abdominal pain and leprosy [4].

**Corresponding author: Email: dralagbe1@finmail.com;*

In view of the abundant potentials in *Indigofera spp*, this study was aimed at analysis of bioactive constituents of *Indigofera zollingeriana* stem bark methanol subjected to gas chromatography- mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1 Experimental Site and Collection of Plant Material

The stem bark of *Indigofera zollingeriana* was collected from Sumitra Research Institute, Gujarat India. It was identified and authenticated by Dr. Xing Liu of the Department of Crop Science, Sumitra Research Institute, India. *Indigofera zollingeriana* stem bark was cut into pieces and air dried for 16 days at 25^{o}C to maintain the bioactive constituents in the plant and pulverized into powder using laboratory blender (Panasonic: Model 34V-Ih).

2.2 Preparation of Extract

50 gram of grinded stem bark of *Indigofera zollingeriana* was dissolved in 250 ml of methanol solvent for extraction using Soxhlet apparatus for 24 hours. The solvent was evaporated by rotary vacuum evaporator to yield a semi solid mass which was later stored in the refrigerator at 4^{o}C for further analysis.

2.3 GC-MS Analysis

GC-MS analysis of methanolic stem bark extract of *Indigofera zollingeriana* was carried out using a Perkin-Elmer GC clarus 500 system and gas chromatograph interfaced to a mass spectrometer equipped with an Elite-I fused silica capillary column (30m × 0.25 mm × ID × 1µm). Injection temperature was maintained at 25^{o}C, helium flow rate as 1.5ml/min and ion source temperature at 230^{o}C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Identifications of the compounds were based on mass spectral matching with standard compounds in National Institute of Standard and Technology (NIST) having more than 62000 patterns.

3. RESULTS AND DISCUSSION

The bioactive chemicals in *Indigofera zollingeriana* stems bark extract reveals that it contains 19 phytoconstituents such as Quercetin-3-O glucosidase (3.44 %), hetranthin A (6.02 %), kaempferol 7-alloside (1.10 %), endecaphyllin (1.40 %), indigoidin (12.49 %), indigotin (10.11 %), 12-Oleanen-3,11-dione (2.50 %), indigoferabietone (21.67 %), 3-nitropropanoic acid (0.77 %), indigo, 2,2-bisindole (14.35 %), cis-(6αβ, 12αβ)-hydroxyrotenone (3.75 %), 12-Oleanen-13,11-dione (2.04 %), methyl 11-octadecenoate (1.71 %), isoliquiritigenin (0.45 %), pentane,1,3-epoxy-4methyl (3.93 %), 3-butyn-2-ol (5.16 %), 9-octadecanoic acid (4.70 %), hexadecanoic acid (2.23 %) and eicosane (1.88 %). The presence of these phytoconstituents will allow plant to perform several activities such as antimicrobial, antiviral, anti-inflammatory, anti-helminthic, antidiuretic and antioxidant effects [6,7]. Hetranthin A, indigoidin, kaempferol 7- alloside and indigotin are group of flavonodial compounds which can be used as adjuvants in vaccine production and are ability to scavenging free radicals, thus protecting disease in animals [8,9,10]. Other groups of compounds identified in the extracts are capable of inhibiting the growth of pathogenic organism and having antiviral properties [11,12].

Table 1. GC-MS analysis of *Indigofera zollingeriana* stems bark extract

Compounds	Molecular formula	Molecular.wgt(g/mol)	Peak area (%)	Retention time
Quercetin-3-O glucosidase	$C_{21}H_{19}O_{12}$	463.4	3.44	9.21
Hetranthin A	$C_{25}H_{16}O_{13}$	0.00	6.02	13.16
Kaempferol 7- alloside	$C_{21}H_{20}O_{11}$	448.4	1.10	13.73
Endecaphyllin	$C_{18}H_{24}N_4O_8$	584.4	1.40	15.74
Indigoidin	$C_{10}H_8N_4O_4$	248.198	12.49	10.33
Indigotin	$C_{16}H_{10}N_2O_2$	262.27	10.11	9.67
12-Oleanen-3,11-dione	$C_{30}H_{46}O_2$	438.7	2.50	10.12
Indigoferabietone	$C_{23}H_{30}O_5$	386.5	21.67	17.38
3-nitropropanoic acid	$C_3H_5NO_4$	119.08	0.77	19.80
Indigo, 2,2-bisindole	$C_8H_{18}O_3$	106.7	14.35	20.57
Cis-(6αβ, 12αβ)-hydroxyrotenone	$C_{23}H_{22}O_7$	410.4	3.75	25.41
12-Oleanen-13,11-dione	$C_{30}H_{46}O_2$	438.7	2.04	24.33
Methyl 11-octadecenoate	$C_{19}H_{36}O_2$	296.5	1.71	27.06

Compounds	Molecular formula	Molecular.wgt(g/mol)	Peak area (%)	Retention time
Isoliquiritigenin	$C_{15}H_{12}O_4$	256.25	0.45	27.55
Pentane,1,3-epoxy-4methyl	$C_6H_{12}O$	100	3.93	29.10
3-butyn-2-ol	C_4H_6O	70	5.16	29.55
9-octadecanoic acid	$C_{18}H_{34}O_2$	282	4.70	30.80
Hexadecanoic acid	$C_{16}H_{32}O_3$	256.4	2.23	30.51
Eicosane	$C_{20}H_{42}O$	282.54	1.88	33.61

4. CONCLUSION

The result shows that *Indigofera zollingeriana* stems bark extract contains several phytochemicals which are precursors for chemo-pharmaceutical semi-synthesis and produce a definite physiological action in the body of animals. The use of plants in animal production prevents the risk of antimicrobial resistance, environmental pollution and toxicity in animal products (milk, eggs and meat). It can also be used to scavenge free radicals; thus preventing diseases and strengthening the immune system.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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