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Isolation, Characterization and Biological activities of ethyl acetate extract of *Heliotropium indicum* (whole plant).

Adebusuyi*, T. A. and Dosumu, O. O.

Department of Industrial Chemistry, University of Ilorin, Ilorin, Nigeria

Abstract

Fresh whole plant of *Heliotropium indicum* was collected, air dried, pulverized and successively extracted with n-hexane, ethyl acetate and ethanol to obtain n-hexane, ethyl acetate and ethanol extracts respectively. The phytochemical screening of the extracts revealed the presence of glycosides, cardiac glycosides, tannins, terpenoids and polyphenols in all the three extracts. Alkaloids, saponins, flavonoids and steroids were present in ethyl acetate and ethanol extracts. The antimicrobial sensitivity tests of the crude extracts on three gram positive bacteria, five gram negative bacteria and five fungi showed significant inhibition zones against most of the tested organisms. The isolated compound from the ethyl acetate extract was characterized with Fourier Transform Infrared Spectroscopy and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and data matching with standard in literature.

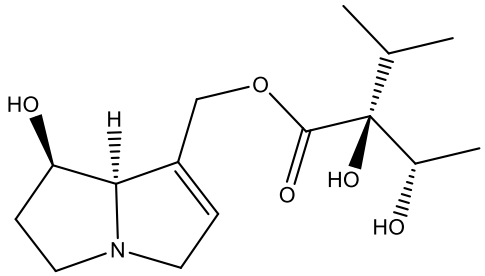
Keywords: *Heliotropium indicum*, phytochemical, isolation, antimicrobial activity.

1. Introduction

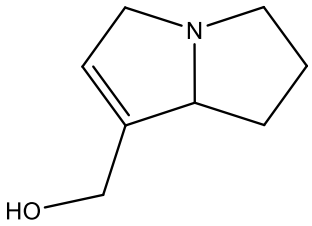
Medicinal plants contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 2008). Recent resistance of diseases to drugs has led to the rise in research on herbal medicine. Phytochemicals in plants has been shown to be responsible for their therapeutic purposes (Cowan, 1999). *Heliotropium indicum* is an annual plant which grows in all parts of Nigeria. It belongs to the family (Boraginaceae) with high medicinal values of the aerial parts (Shoge *et al*., 2011), leaves, the stem and the roots (Akinlolu *et al*., 2008). Birecka *et al*. (1984) reported that *Heliotropium indicum* contains the pyrrolizidine alkaloids indicine, indicine -N- oxide, acetyl - indicine,

*Corresponding Author: Adebusuyi, T. A.
Email: tos4olopa@gmail.com

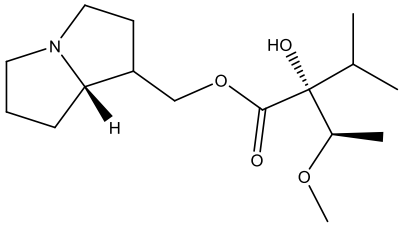
indicinine, lycopsamine (**1**), heliotrine, supinine, supinidine (**2**), heleurine (**3**) and lindelofidine, all of which are thought to have hepatotoxic activity.



Lycopsamine (**1**)



Supinidine (**2**)



Heleurine (**3**)

Akinlolu *et al*., (2008), reported that the histo-gastroprotective potential of the aqueous extract of the dried leaves of *Heliotropium indicum* against indomethacin-induced ulceration in rats might in part be due to the presence of phytochemicals particularly; tannins, alkaloids and saponins.

This research reports the antimicrobial study on *Heliotropium indicum* and isolation of ethyl hexadecanoate which has been shown to possess antioxidant, antiandogenic, flavour, pesticidal, lubricating and hemolytic properties (Sermakkani *et al*., 2012).

2. Materials and methods

2.1 Sample collection and treatment

Fresh plant of *Heliotropium indicum* was collected from Omupo in Kwara state Nigeria. The plant was authenticated at the herbarium of the Department of Plant Biology, University of Ilorin, and specimen copy with herbarium number UIH001/1108 was deposited. It was air dried at room temperature, crushed and pulverized prior to extraction.

2.2 Extraction procedures

400g of the pulverized sample was kept in contact with n-hexane in a stoppered glass container for three days with frequent agitation (Ncube *et al*., 2008). The extract was decanted and filtered, after which the remains was air dried. The filtrate from the n-hexane extraction was concentrated under vacuum using rotary evaporator. The dried concentrate was stored in an opaque glass bottle and kept in the refrigerator for further studies. The air dried remains was placed in another stoppered glass container and ethyl acetate was added to it for three days with frequent agitation. The crude extract was filtered, while the remains was once again air dried and the ethyl acetate filtrate concentrated. A subsequent extraction using the above procedure was use to obtain ethanol extract. This method is known as successive extraction by maceration.

2.3 Phytochemical test

Phytochemical screening was carried out on the n-hexane, ethyl acetate and ethanol extracts using standard procedures to identify the class of secondary metabolites present in the plant extracts (Harborne, 1973; Trease and Evans, 1989 and Sofowora, 1993). Alkaloids, saponins, phlobatannins, flavonoids, steroids, terpenoids, cardiac glycosides, tannins, glycosides, polyphenols and anthraquinones were tested for.

2.4 Antimicrobial sensitivity test

The antimicrobial sensitivity test was carried out on the crude extracts in order to know the extent to which the crude extracts can inhibit the growth of the tested pathogenic microorganisms. The antibacterial tests were carried out using pour plate method (Oloyede and Onocha, 2010) while the antifungal tests was done using surface plate method (Bayer *et al*., 1986). The zones of inhibition were measured and recorded. The test was carried out in triplicate and the average was calculated with their standard deviation.

2.5 Thin Layer Chromatography (TLC)

TLC was used to determine the number of chemical components in the ethyl acetate extract and the most suitable mobile phase for good resolution for components isolation by column chromatography. The plant extract was dissolved in minimal amount of ethyl acetate, spotted at the base of the TLC plate and developed using n-hexane, ethyl acetate and ethanol in different ratios. The resulting chromatogram, after air-drying was viewed under short wavelength (254nm) and long wavelength (366nm) ultraviolet light. Finally, a ratio of 4:1 of

n-hexane and ethyl acetate was found to be the most appropriate mobile phase that provided a good.

2.6 Column chromatography

20g slurry of crude ethyl acetate extract was loaded on the column of length 100cm and diameter 5cm. Elution was started with 100% n-hexane and polarity was gradually increased by gradient addition of ethyl acetate. A total of 70 fractions were obtained.

2.7 Preparative thin layer chromatography

Self-coated preparative thin layer chromatography plates were used to purify the isolated chemical components. The plates were coated with slurry of silica gel mixed with appropriate amount of binder (calcium sulphate) and activated at 120°C for 15mins in a drying oven.

2.8 Isolation of active component

The isolation of an active component present in the ethyl acetate extract was carried out using Column Chromatography packed with Silica gel (70 – 230 mesh, merek). The isolates were purified with Preparative Thin Layer Chromatography.

2.9 Spectroscopic analysis of the isolated compounds

The purified isolated compounds were analyzed using Perkin Elmer Fourier Transform Infrared (FTIR) spectrometer at the Multidisciplinary Central Research Laboratory of University of Ibadan, Ibadan, Oyo State, Nigeria while Gas Chromatography-Mass Spectroscopic analysis was carried out with Agilent 7890 GC-MS at the Federal University of Technology Akure, Ondo State, Nigeria.

3. Results and Discussion

3.1 Phytochemical Screening

Phytochemical screening carried out on the n-hexane, ethyl acetate and ethanol extracts revealed the presence of glycosides, cardiac glycosides, tannins, terpenoids and polyphenols in the whole plant of *Heliotropium indicum* with the exception of ethanol extract where polyphenolics was not detected (Table 1). Alkaloids, saponins, flavonoids and steroids were present in both ethyl acetate and ethanol extracts. Anthraquinones and phlobatannins though tested for were not detected in any of the three extracts of *Heliotropium indicum*. The

phytochemicals present in plants are known to be responsible for the therapeutic benefits of medicinal plants (Shoge *et al*., 2011). The wound healing activity of this plant in folk medicine is supported by the presence of phytochemicals such as tannins which have been shown to possess wound healing properties (Shoge *et al*., 2011).

Table 1: Result of phytochemical screening of *Heliotropium indicum* extracts

Phytochemicals	HIH	HIEa	HIEt
Saponins	-	+	+
Flavonoids	-	+	+
Terpenoids	+	+	+
Tannins	+	+	+
Polyphenols	+	+	-
Cardiac glycosides	+	+	+
Phlobatannins	-	-	-
Alkaloids	-	+	+
Steroids	-	+	+
Anthraquinones	-	-	-
Glycosides	+	+	+

HIH - *Heliotropium indicum* n-hexane extract;
HIEa - *Heliotropium indicum* ethylacetate extract;
HIEt - *Heliotropium indicum* ethanol extract. **- = absent** **+ = present**

3.2 Antimicrobial test

The result of antimicrobial sensitivity test of the n-hexane, ethyl acetate and ethanol extracts are shown in Table 2. It showed that ethyl acetate and ethanol extracts were more active than the n-hexane extract which showed lower zones of inhibition on the tested microorganisms. The effect of ethyl acetate and ethanol extracts were pronounced on *Staphylococcus aureus,*

Salmonella typhi, *Candids albieans*, *Candids krusei*, *Aspergillus niger* and *Tricophyton rubrum* by the showing of larger diameter of inhibition zones (DIZ) as compared to that of n-hexane extract. The n-hexane extract showed it greatest activity on *Bacillus subtilis*, while all three extracts have similar effect on *Proteus mirabilis* i.e. same diameter of inhibition at the same concentration of extracts. This inhibition activity is corroborated by the earlier work of Cowan, (1999) which showed that the presence of phytochemicals like tannins, terpenoids, flavonoids and alkaloids in the extracts are responsible for antimicrobial activities.

Table 2: Antimicrobial activity of *Heliotropium indicum* extracts

Test organisms	DIZ of N-hexane extract (mm)						DIZ of ethylacetate extract (mm)						DIZ of ethanol extract(mm)						Control	
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F	-	+
E. coli	18	14	12	10	0	0	20	18	14	12	10	0	18	14	12	10	0	0	0	38
S. aureus	16	14	12	10	0	0	24	20	18	16	14	10	26	24	20	18	14	10	0	38
P. aeruginosa	16	14	10	0	0	0	18	14	12	10	0	0	16	12	10	0	0	0	0	36
B. subtilis	18	16	12	10	0	0	16	14	12	10	0	0	14	12	10	0	0	0	0	38
S. typhi	14	12	10	0	0	0	16	14	12	10	0	0	18	16	12	10	0	0	0	38
K. pneumonia	16	14	12	10	0	0	16	14	12	10	0	0	14	12	10	0	0	0	0	38
P. mirabilis	18	14	12	10	0	0	18	14	12	10	0	0	18	14	12	10	0	0	0	36
S. viridians	18	14	12	10	0	0	20	18	16	14	12	10	18	16	12	10	0	0	0	38
C. albicans	14	12	10	0	0	0	16	14	12	10	0	0	16	14	12	10	0	0	0	28
C. krusei	12	10	0	0	0	0	16	14	12	10	0	0	14	12	10	0	0	0	0	26
A. niger	12	10	0	0	0	0	14	12	10	0	0	0	14	12	10	0	0	0	0	28
R. stolonifer	14	12	10	0	0	0	14	12	10	0	0	0	16	12	10	0	0	0	0	26
T. rubrum	14	12	10	0	0	0	18	14	12	10	0	0	18	14	12	10	0	0	0	28

DIZ – Diameter of inhibition zone; A – 200µg/ml of extract; B – 100µg/ml of extract; C – 50µg/ml of extract; D – 25µg/ml of extract; E – 12.5µg/ml of extract; F – 6.25µg/ml of extract; -ve – negative control (DMSO); +ve – positive control (Gentamycin 10µg/ml for bacteria, Tioconazole 70% for fungi)

3.3 Spectroscopic analysis results of the isolated compound from ethyl acetate extract

3.3.1 FTIR analysis of isolated compounds

The bands observed from the FTIR spectra of the isolated compound from ethyl acetate extract are shown in Figure 1 and important functional group absorptions are shown in Table 3.

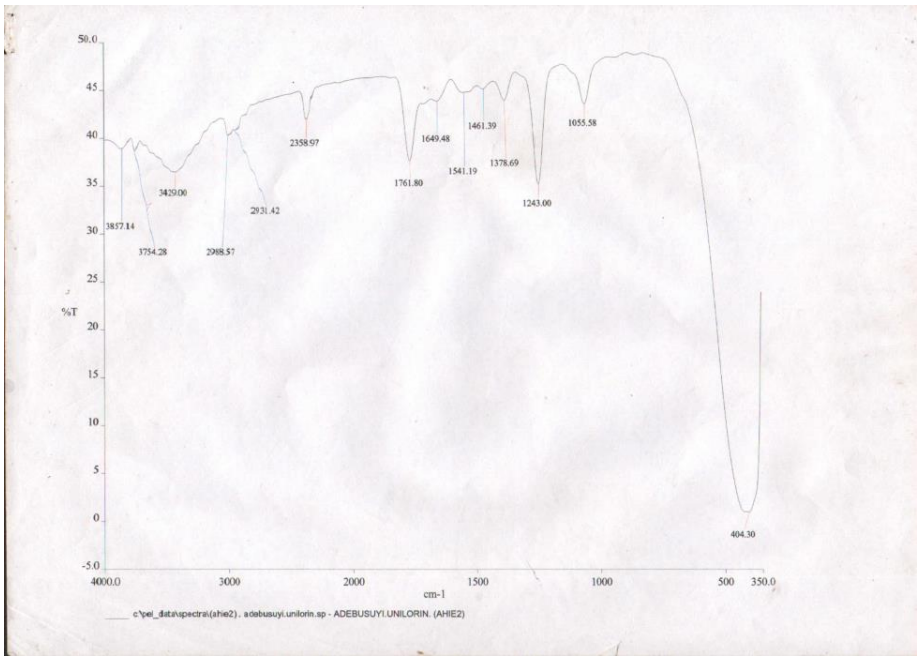


Figure 1: FTIR spectrum of AHIE2

Table 3: Characteristic FTIR data of the isolated compounds

S/No	Sample code	υ_{OH} (cm^{-1})	$\upsilon_{C-H\ Aliphatic}$ (cm^{-1})	$\upsilon_{C=O}$ (cm^{-1})	υ_{C-O} (cm^{-1})	υ_{C-H}^{bend} (cm^{-1})
1	AHIE2	3429.00	2988.57 2931.42	1761.80	1243.00	1461.39 1378.69

3.4.2 GC-MS analysis result of AHIE2

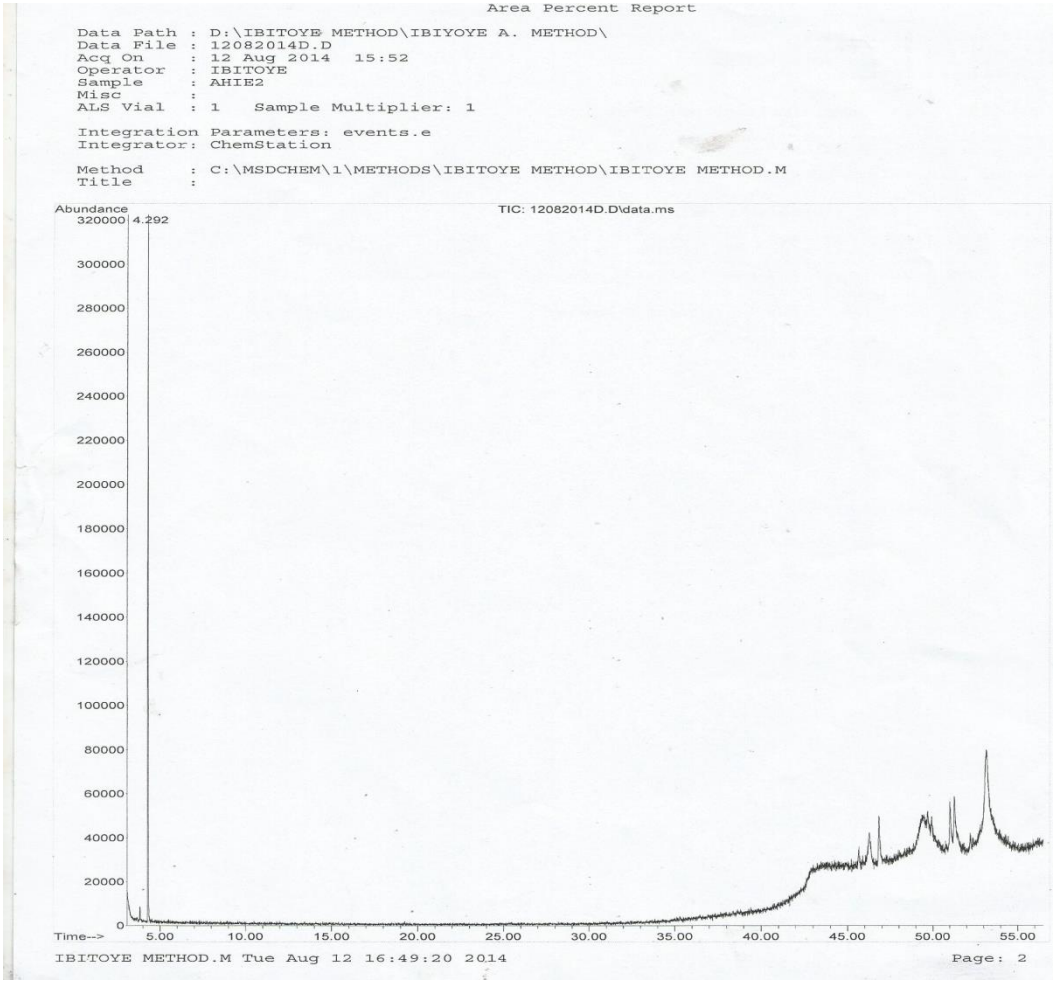


Figure 2: GC-MS spectrum of AHIE2

The main peak highlighted in the GC-MS is shown in Table 4.0.

Table 4.0: GC-MS analysis result of AHIE2

S/No	Name of proposed compound	Retention Index	% Composition	Mass spectra fragmentation pattern
1	Hexadecanoic acid, ethyl ester	1978	100	55, 69, 88,101,115, 129, 143, 157, 171, 185, 199, 213, 239, 255,284.

The GC-MS result of AHIE2 revealed a major peak indicating that only one major compound is present in the isolated compound. Comparison with National Institute of Standards and Technology library hits showed that the isolated compound is hexadecanoic acid, ethyl ester (100%) with a retention time of 4.292 minutes. The ester with IUPAC name ethyl hexadecanoate, has molecular formula of $C_{18}H_{36}O_2$, molar mass of 284 and base peak of 88. The fragmentation pattern of the compound is proposed below:

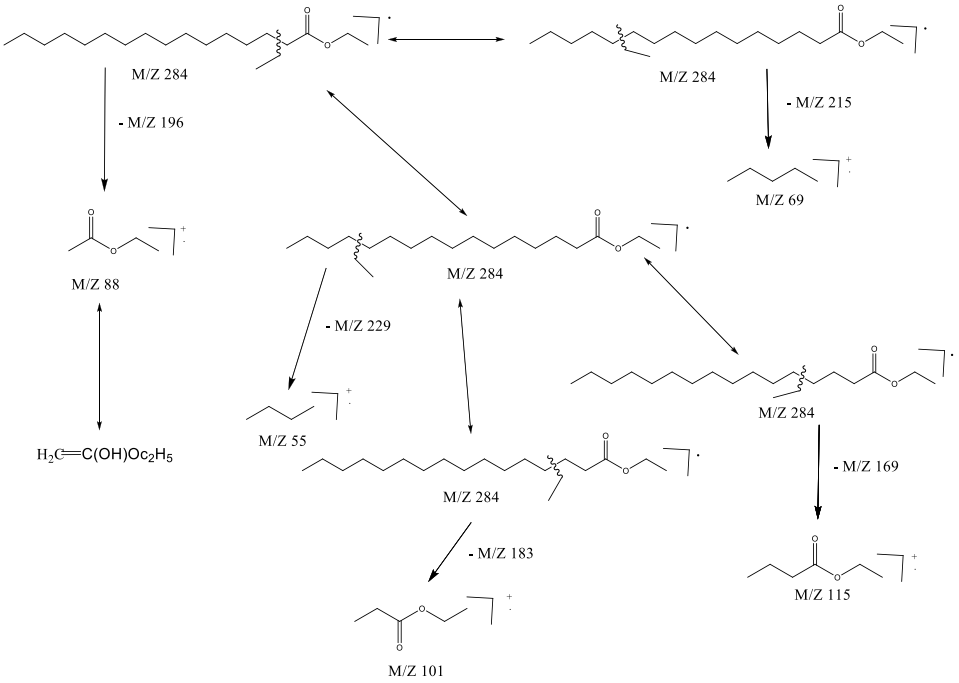


Figure 3: Proposed fragmentation pattern of ethyl hexadecanoate.

Ethyl hexadecanoate which is a fatty acid ester has earlier been shown to possess antioxidant and antimicrobial properties (Bodoprost *et al*., 2007). It has also been shown to possess antiandogenic, flavour, pesticidal, lubricating and hemolytic properties (Sermakkani *et al*., 2012) which is responsible for it medicinal use in folk medicine.

4. Conclusion

Ethyl hexadecanoate was isolated in its pure form from ethyl acetate extract of *Heliotropium indicum* in this research work. The antimicrobial properties of this compound as reported in literature can serve as a new lead for viable drug synthesis. Also, the presence of

phytochemicals such as alkaloids, tannins, saponins and glycosides in ethyl acetate extracts of the plant confirms the medicinal properties of *Heliotropium indicum* as reported by Shoge *et al*., 2011. Isolation of these phytochemicals should be carried out using high performance of liquid chromatography to obtain better result.

Acknowledgement

Chemistry Department Research Laboratory, University of Ilorin, University of Ibadan, Central Research Laboratory, Federal University of Technology Akure, Research Laboratory.

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