International Journal of Science and Engineering Applications
Volume 4 Issue 5, 2015, ISSN-2319-7560 (Online)

Acaricidal Activity of *Ethulia conyzoides* Extracts and Constituents against *Tetranychus urticae* Koch

A. Mahmoud Dawidar
Chem. Dept., Fac. of Sci.,
Mansoura University
Mansoura, Egypt

M. Abdel-Mogib
Chem. Dept., Fac. of Sci.,
Mansoura University
Mansoura, Egypt

M. El Saied. El-Naggar
Plant Protection Research
Institute, A.R.C., Egypt

M. El-Hoseiny Mostafa
Plant Protection Research
Institute, A.R.C., Egypt

Abstract: The acaricidal potential of *Ethulia conyzoides* L. aerial parts extracts as well as its isolated compounds were investigated against larvae and adults of *Tetranychus urticae* Koch under laboratory conditions. Chromatographic separation of the extracts gave 3-O-acetyl lupeol, lupan-3-ol, ethuliacoumarin (**1**) and 7-O-methyl apigenin (Genkwanin), besides a mixture of isoethyliacoumarin A (**2**), isoethuliacoumarin C (**3**). The biological evaluation results indicated that ethyl acetate fraction and ethuliacoumarin (**1**) were the most potent to larvae and adults of *Tetranychus urticae*. The LC_{50} values of ethyl acetate fraction were found to be 11.58 and 17.86 ppm, respectively and LC_{50} values of (**1**) were found to be 12.72 and 19.22 ppm, respectively after 7 days of treatment.

Keywords: Acaricidal activity, *Tetranychus urticae* Koch, *Ethulia conyzoides*, 3-O-acetyl lupane, lupan-3-ol, ethuliacoumarin, 7-O-methyl apigenin, isoethyliacoumarin A and isoethuliacoumarin C.

1. INTRODUCTION

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a worldwide damaging pest attacking cotton, fruit trees and vegetables in Egypt [1]**.** Synthetic chemical acaricides has been the major tool in pest control operations. However, the extensive and repeated use of synthetic organic pesticides have led to environmental problems to the human health, non-target organisms and cause some disastrous ecological damage [2].

The move towards green pesticides represents a new trend for discovering natural pesticides. Natural products considered to be an excellent alternative to synthetic pesticides as a mean to reduce negative impacts to human health and the environment [3].

Ethulia conyzoides L., a wild growing Egyptian plant, has for centuries been used in folk medicine as an anti-helminthic for round worms and for abdominal disorders [4, 5], a source of natural antioxidants [6] and antihypertensive agents. It was used to cure headaches and dysmenorrhea [7]. In addition, the methanol extract of the aerial parts of *E. conyzoides* has antibacterial activity [5].

The molluscicidal activity of *E. conyzoides* was recorded [4], which was attributed to the presence of ethuliacoumarin A and isoethuliacoumarin A.

The previous phytochemical screening revealed that *E. conyzoides* contains terpenoid 5-methylcoumarins [5, 8, 9, 10, 11, 12], triterpenoids [13, 14], flavonoids [15] and sterols [13].

This article presents the results of phytochemical and acaricidal activity investigation of *E. conyzoides.*

2. MATERIALS AND METHODS

2.1 General

NMR experiments were performed on a Bruker AMX 400 instrument standard pulse sequences operating at 400 MHz in ^{1}H-NMR and ^{13}C-NMR. Chemical shifts are given in δ (ppm) relative to TMS as internal standard material and the coupling constants (*J*) are in Hz.

GC/MS analysis of the volatile fractions were performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components, at Agriculture Research Center, Dokki, Cairo. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter) coated with poloy dimethyl-siloxane (0.5 µm. film thickness). The oven temperature was programmed from 50°C for 3 min., at isothermal, then heating by 7°C /min. to 250°C and isothermally for 10 min., at 250°C. Injector temperature was 200°C and the volume injected was 0.5 µl. Transition-line and ion source temperatures were 250°C and 150°C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV.

2.2 Chemicals

Normal CC: column chromatography was performed on silica gel Merck grain size 0.2-0.063 mm; preparative TLC were performed on silica gel

(Kieselgel 60, F 254) of 0.25 mm thickness. Pet. ether (60-80℃), methylene chloride, ethyl acetate and methanol were obtained from Adwic Company.

2.3 Plant material

The aerial parts of *Ethulia conyzoides* L. was collected on canal banks near Mansoura in April, 2009, identified by Prof. Dr. Loutfy Boulos, Professor of Botany, Faculty of Science, Alexandria University, Egypt.

2.4 Extraction and isolation

The aerial parts of *E. conyzoides* (1 Kg) were extracted by soxhlet apparatus using different organic solvents of different polarities composed of pet. ether- methylene chloride- ethyl acetate and methanol successively. In order to obtain four fractions, pet. ether fraction (20.40 g), methylene chloride fraction (9.28 g), ethyl acetate fraction (3.05 g) and methanol fraction (53.72 g), which was further extracted by butanol to give the butanol fraction (2.31 g).

E. conyzoides fresh aerial parts (250 g) were processed in order to obtain the volatile oil fraction by means of hydro-distillation technique (0.54 g, 0.22% fresh weight).

2.5 Separation of compounds

The pet. ether fraction (9.45g) was defatted using cold methanol to give the defatted pet. ether fraction (9.42 g). The defatted pet ether residue was subjected to column chromatography using silica gel as adsorbent. Elution of the column was performed by using a series of eluents from hexane/ acetone combinations of increasing polarity. The effluents were combined into four sub-fractions according to their TLC patterns.

Sub-fraction 1 a yellow orange oily material which was eluted by hexane/ acetone (4: 1) was further purified using preparative TLC eluted by (hexane / ether 3:1) to yield white needle crystals, R_f = 0.69 of 3-O-acetyl lupeol.

Pet. ether fraction afforded by GC/MS technique 6 compounds as indicated by table 1.

Methylene chloride fraction (9.28 g) was chromatographed over silica gel CC using a series of eluents from pet. ether / ethyl acetate combinations of increasing polarities. The effluents were recombined into eight sub-fractions based on their TLC patterns.

The sub-fraction 2 eluted by pet. ether / ethyl acetate (13:7) gave by preparative TLC (silica gel, benzene / ether 4 : 1) lupan-3-ol at R_f = 0.28 and compound (**1**) at R_f = 0.53.

The sub-fraction 3 was purified using preparative TLC (silica gel, $CHCl_3$ / MeOH (39: 1)) to afford a mixture of compounds (**2**) and (**3**) at R_f = 0.19.

Methylene chloride fraction afforded by GC/MS technique 5 compounds as indicated by table 1.

The ethyl acetate fraction (3.05 g) was purified on sephadex-LH-20, which was washed with MeOH, the effluents were combined into five sub fractions based on their TLC patterns.

Sub-fraction 5 was further purified using preparative TLC developed in a mixture of benzene / acetone (3:2), where 7-*O*-methyl apigenin was obtained at R_f = 0.67.

The volatile oil fraction (0.54 g, 0.22%) was separated by GC/MS to afford 8 compounds.

2.6 Maintance of spider mite colony

Colony of spider mite *Tetranychus urticae* Koch was reared under laboratory condition (25±2 °C and 60±5 % R.H) at plant protection research institute branch, Dakahlia Governorate. This study colony was isolated from heavily infested castor oil plant leaves. Spider mite colony was reared on castor oil leaves. These leaves were cleaned and placed on moisten cotton wool pad in Petri dishes. This colony was left for one year under the precious conditions in order to get a homogenous and sensitive colony. Spider mite individual were transferred to the leaves by the aid of fine camels hair brush. Breeding leaves were changed twice weekly at summer and once weekly at winter. Adding water was done twice daily to prevent escaping of *T. urticae* individuals.

2.7 Assessment of acaricidal activity

In this respect, laboratory experiments are conducted to evaluate the activity of various tested plant extracts against *T. urticae* mobile stages (larvae and adult females). The leaf-dip technique was used Gazal *et al*., (1992) [16].

The indication of mortality was chosen as the failure of mites to respond positively by leg movement followed light brooding with a fine brush. Mortality percentages were determined and corrected by using Abotts, (1925) formula [17] and they are statistically analyzed to estimate LC_{50}, LC_{90} and slope values according to Finney, (1971) [18]. Toxicity index was computed for different extracts and their isolated compounds by comparing these materials with the most effective extracts or isolated compounds using Sun's, (1950) equation [19].

$$\text{Toxicity index} = \frac{LC_{50} \text{ of compound A}}{LC_{50} \text{ of compound B}} \times 100$$

Where:

A is the most effective compound

B is the tested compound

3. RESULTS AND DISCUSSION

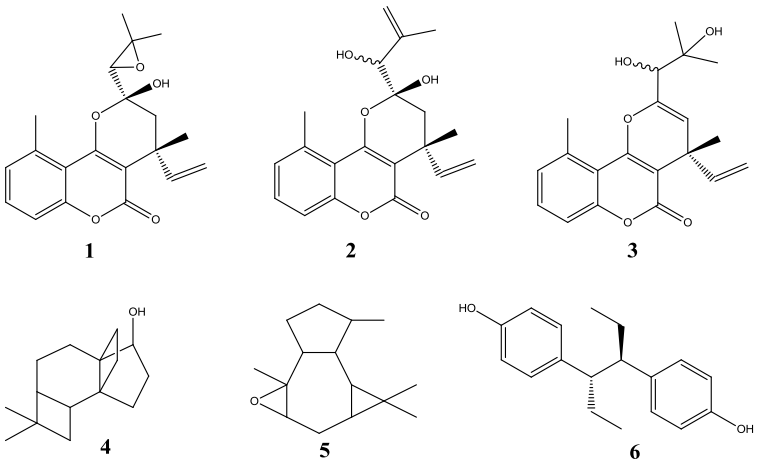
The chromatographic separation of the plant extracts and the spectral analysis of the separated compounds revealed the identification of the triterpenoids lupyl acetate [20] and was previously isolated from the same plant species [13] and lupeol [21]) that was isolated before from *E. conyzoides* [13], The monoterperpenoidal 5-methylcoumarins ethuliacoumarin (1) which was isolated previously from the same plant species by Baldaa *et al*., (1980) and Mahmoud *et al*., (1998) [8, 9], isoethyliacoumarin A (2) and isoethuliacoumarin C (3) which were isolated previously from the same plant species by (Baldaa *et al*., 1980) [8]**,** as well as the flavonoid 7-*O*-methyl apigenin (**Genkwanin**) [22], which has not been isolated previously from *E. conyzoides*.

The pet ether, methylene chloride and the volatile oil fractions of the aerial parts of *E. conyzoides* were

analyzed by GC/MS technique. Nineteen compounds which are listed in table 1 were identified.

Table 1: Chemical constituents identified in fractions of *E. conyzoides* aerial parts by GC/MS technique.

Component name	R_t, min	Area %	M.F	(Identity) m/z (ret. int. %)
Pet. ether fraction				
4,4-dimethyl-Tetracyclo[6.3.2.0 (2,5).0(1,8)]tridecan-9-ol (**4**)	15.17	0.80	$C_{15}H_{24}O$	220 (1) $[M^+]$, 205 (3) $[M- CH_3]^+$, 187 (7) $[M- CH_3- H_2O]^+$, 163 (10) $[C_{11}H_{15}O]^+$, 136 (100) $[C_9H_{12}O]^{\cdot+}$, 91 (37) $[C_7H_7]^+$, 65 (20) $[C_5H_5]^+$
Isoaromadendrene epoxide (**5**)	15.63	0.43	$C_{15}H_{24}O$	220 (7) $[M^+]$, 205 (13) $[C_{15}H_{25}]^+$, 149 (43) $[C_{11}H_{17}]^+$, 134 (96) $[C_{10}H_{14}]^{\cdot+}$, 105 (98) $[C_8H_9]^+$, 91 (100) $[C_7H_7]^+$, 77 (67) $[C_6H_5]^+$, 65 (27) $[C_5H_5]^+$
Perhydrofarnesyl acetone	17.80	1.17	$C_{18}H_{36}O$	268 (1) $[M^+]$, 250 (13) $[C_{18}H_{34}]^+$, 225 (1) $[C_{16}H_{33}]^+$, 109 (35) $[C_8H_{13}]^+$, 85 (35) $[C_6H_{13}]^+$, 71 (63) $[C_5H_{11}]^+$, 58 (100) $[C_3H_6O]^+$
2,3,5-trimethyl-7H-Furo[3,2-g][1]benzopyran-7-one	20.76	2.53	$C_{14}H_{12}O_3$	228 (1) $[M^+]$, 227 (3) $[M- H]^+$, 213 (7) $[M- CH_3]^+$, 199 (10) $[M- H- CO]^+$, 185 (100) $[M- CH_3- CO]^+$, 175 (100) $[M- CH_3- CO- CO]^+$, 91 (37) $[C_7H_7]^+$, 77 (67) $[C_6H_5]^+$
Phytol isomer	21.08	1.50	$C_{20}H_{40}O$	297 (1) $[M^{+\cdot}]$, 123 (74) $[C_9H_{15}]^+$, 95 (78) $[C_7H_{11}]^+$, 71 (100) $[C_5H_{11}]^+$, 55 (63) $[C_4H_7]^+$.
Stigmasterol	35.58	3.74	$C_{29}H_{48}O$	412 (100) $[M^+]$, 394 (13) $[M- H_2O]^+$, 300 (43) $[C_{21}H_{32}O]^+$, 255 (54) $[C_{19}H_{27}]^+$, 231 (12) $[C_{16}H_{23}O]^+$, 213 (30) $[C_{16}H_{21}]^+$, 133 (43) $[C_{10}H_{13}]^+$, 55 (70) $[C_4H_7]^+$
Methylene chloride fraction				
Benzaldehyde,4-methoxy	12.33	1.90	$C_8H_8O_2$	136 (100) $[M^+]$, 135 (100) $[M^+- H]^+$, 107 (13) $[M^+- CO]^+$, 105 (7) $[M^+- OCH_3]^+$, 77 (20) $[C_6H_5]^+$
Coniferyl alcohol	16.56	0.54	$C_{10}H_{12}O_3$	180 (74) $[M^+]$, 164 (100) $[M^+-OCH_3]^+$, 137 (100) $[C_8H_9O_2]^+$, 124 (53) $[C_7H_8O_2]^{\cdot+}$, 91 (32) $[C_7H_7]^+$, 77 (20) $[C_6H_5]^+$
(-)-Loliolide	17.04	1.45	$C_{11}H_{16}O_3$	196 (26) $[M^+]$, 178 (9) $[M^+- H_2O]^+$, 163 (13) $[M^+- H_2O-CH_3]^+$, 135 (48) $[C_9H_{11}]^+$, 111 (100) $[C_6H_7O_2]^+$, 91 (78) $[C_7H_7]^+$, 65 (9) $[C_5H_9]^+$.
Neophytadiene	18.02	0.61	$C_{20}H_{38}$	278 (9) $[M^+]$, 123 (65)$[C_9H_{15}]^+$, 95 (88) $[C_{17}H_{11}]^+$, 82 (77) $[C_6H_{10}]^+$, 71 (4) $[C_5H_{11}]^+$, 68 (100) $[C_5H_8]^+$, 55 (84) $[C_4H_7]^+$
Phytol	21.05	0.36	$C_{20}H_{40}O$	297 (4) $[M^{+\cdot}]$, 123 (28) $[C_9H_{15}]^+$, 95 (13) $[C_7H_{11}]^+$, 71 (100) $[C_5H_{11}]^+$, 55 (27) $[C_4H_7]^+$.
volatile oil fraction				
Trans-caryophyllene	11.89	0.43	$C_{15}H_{24}$	204 (13) $[M^+]$, 189 (33) $[M-CH_3]^+$, 147 (40) $[C_{11}H_{15}]^+$, 133 (100) $[C_{10}H_{13}]^+$, 91 (86) $[C_7H_7]^+$, 55 (40) $[C_4H_7]^+$,
(-)-Caryophyllene oxide	14.43	1.30	$C_{15}H_{24}O$	220 (1) $[M^+]$, 205 (7) $[M-CH_3]^+$, 149 (17) $[C_{11}H_{17}]^+$, 123 (17) $[C_9H_{15}]^+$, 109 (50) $[C_7H_9O]^+$, 93 (86) $[C_7H_9]^+$, 55 (40) $[C_4H_7]^+$.
Caryophylla-4(12),8(13)-dien-5β-ol	15.17	0.47	$C_{15}H_{24}O$	220 (1) $[M^+]$, 205 (2) $[M-CH_3]^+$, 187 (3) $[M-CH_3-H_2O]^+$, 136 (100) $[C_{10}H_{16}]^{\cdot+}$, 109 (20) $[C_7H_9O]^+$, 91 (34) $[C_7H_7]^+$.
Hexestrol (**6**)	20.55	0.20	$C_{18}H_{22}O_2$	270 (22) $[M^+]$, 135 (100) $[C_9H_{11}O]^+$, 120 (7) $[C_8H_8O]^+$, 107 (3) $[C_7H_7O]^+$, 77 (15) $[C_6H_5]^+$.
Acetyl tri-n-butyl citrate	22.73	0.46	$C_{20}H_{34}O_8$	329 (7) $[C_{16}H_{25}O_7]^+$, 301 (1) $[C_{15}H_{25}O_6]^+$,259(60) $[C_{12}H_{19}O_6]^+$, 185 (100) $[C_9H_{13}O_4]^+$, 157 (100) $[C_8H_{13}O_3]^+$, 129 (45) $[C_6H_9O_3]^+$.
Taraxasterol	25.28	1.36	$C_{30}H_{50}O$	426 (53) $[M^+]$, 411 (27) $[M- CH_3]^+$, 393 (7) $[M-CH_3-H_2O]^+$, 272 (8) $[C_{20}H_{32}]^{\cdot+}$, 207 (78) $[C_{14}H_{23}O]^+$, 135 (97) $[C_{10}H_{15}]^+$, 95 (72)$[C_7H_{11}]^+$, 77 (17) $[C_6H_5]^+$, 55 (50) $[C_4H_7]^+$.
β-Amyrin acetate	27.52	1.52	$C_{32}H_{52}O_2$	468 (99) $[M^+]$, 393 (49) $[M-CH_3-H_2O]^+$, 218 (48) $[C_{16}H_{26}]^+$, 203 (55)$[C_{15}H_{23}]^+$, 189 (81) $[C_{14}H_{21}]^+$.
Lupeol actate	29.29	2.69	$C_{32}H_{52}O_2$	468 (37) $[M^+]$, 453 (17) $[M- CH_3]^+$, 393 (10) $[M-CH_3-CH_3COOH]^+$, 203 (37) $[C_{15}H_{23}]^+$, 189 (100) $[C_{14}H_{21}]^+$, 161 (28) $[C_{12}H_{17}]^+$,135 (47) $[C_{10}H_{15}]^+$, 121 (47) $[C_9H_{13}]^+$.



The results of acaricidal activity were presented obtained in table (2) that showed the efficiency of *E. conyzoides* fractions and their isolated compounds against the larvae of *T. urticae* after 7 days of treatment. The ethyl actate fraction was the most effective at LC_{50} level followed by butanol fraction, pet. ether fraction, methylene chloride fraction and essential oil extract. The LC_{50} values of these fractions were 11.58, 21.00, 53.63, 76.47 and 282.23 ppm, respectively. Taking the toxicity index into consideration, it could be also observed that ethyl actate fraction was the most effective fraction against the larvae of *T. urticae* after 7-days of treatment followed by butanol fraction, pet. ether fraction, methylene chloride fraction and essential oil extract.

The susceptibility of the adult females of *T. urticae* to various plant fractions after 7-days of treatments (table 2) showed that the LC_{50} values were 17.86, 53.37, 109.73, 175.94 and 334.73 ppm for ethyl acetate fraction, butanol fraction, methylene chloride fraction, pet. ether fraction and essential oil fraction, respectively.

The toxicity index of the LC_{50} values showed that ethyl acetate fraction was the most effective plant fraction against adult females of *T. urticae* after 7-days of treatment, followed by butanol fraction, methylene chloride fraction and pet. ether fraction. Essential oil fraction was the least effect one.

Bioassay-guided fractionation led to the isolation and purification of six compounds which were investigated as acaricides. The toxicity of the isolated compounds of *E. conyzoides* fractions against adult females of *T. urticae* were presented in table (2). Ethuliacoumarin (**1**) was the most effective isolated compound against larvae and adult females of *T. urticae* after 7- days of treatment followed by (isoethyliacoumarin A (**2**), isoethuliacoumarin C (**3**)), lupan-3-ol, 3-O-acetyl lupeol and genkwanin.

Our results were in agreement with the finding by Kady *et al*., (1992) who reported that the molluscicidal principles of *E. conyzoides* were identified as ethuliacoumarin A and isoethuliacoumarin A [4].

Table 2. Toxicity of plant fractions and isolated compounds against larvae and adult females of *T. urticae* after 7- days of treatment.

Plant extract	Larvae				Adult females			
	LC_{50} (ppm) and confidence limits at 95%	**LC_{90} (ppm) and confidence limits at 95%**	**Slope**	**Toxicity index at LC_{50} value**	**LC_{50} (ppm) and confidence limits at 95%**	**LC_{90} (ppm) and confidence limits at 95%**	**Slope**	**Toxicity index at LC_{50} value**
Pet. ether fraction	53.63 20.35 93.05	1225.41 540.97 7983.04	0.943±0.210	21.59	175.94 96.40 273.84	2250.52 1108.31 20981.68	1.104±0.259	10.15
Methylene Chloride fraction	76.47 50.30 105.49	496.28 304.99 1242.25	1.578±0.283	15.14	109.73 38.45 212.01	9095.97 1999.50 14463.1 E+2	0.668±0.189	16.28
Ethyl acetate fraction	11.58 5.69 17.59	130.84 68.55 578.12	1.217±0.270	100.00	17.86 10.26 25.16	100.68 67.54 208.27	1.707±0.320	100.00
Butanol fraction	21.00 4.39 38.37	800.43 245.60 81924.59	0.811±0.254	55.14	53.37 31.37 76.69	442.08 254.23 1373.01	1.396±0.276	33.46
Essential oil fraction	282.23 116.70 466.06	6146.57 2315.09 11439.8E+1	0.958±0.256	4.10	334.73 129.12 532.61	3762.10 1995.07 18461.55	1.220±0.298	5.34
3-O-acetyl lupeol	201.64 100.93 285.45	910.73 628.18 1991.13	1.957±0.447	6.31	348.61 184.21 516.21	3307.25 1848.00 11578.63	1.312±0.275	5.51
lupan-3-ol	137.21 60.66 201.32	774.15 500.10 2160.80	1.706±0.416	9.27	182.19 87.43 274.70	1609.07 951.62 4858.05	1.354±0.286	10.55
Ethuliacoumarin (1)	12.72 3.92 22.24	396.98 128.10 21521.78	0.858±0.254	100.00	19.22 7.20 37.31	863.57 200.97 39855.5E+1	0.776±0.249	100.00
Isoethyliacoumarin A (2), Isoethuliacoumarin C (3)	35.46 8.36 60.72	261.77 171.33 684.08	1.476±0.388	35.87	66.64 44.21 92.41	452.89 271.15 1209.23	1.540±0.279	28.84
Genkwanin	297.55 69.07 489.80	3714.43 1720.12 459176.86	1.169±0.370	4.27	362.79 195.92 573.89	5745.07 2378.06 57914.33	1.068±0.257	5.30

4. REFERENCES

[1] Dawidar, A. M., Abdel-Mogib, M., El-Naggar, M. E., Mostafa, M. E. (2014). Isolation and Characterization of *Polygonum equisetiforme* Flavonoids and Their Acaricidal Activity against *Tetranychus urticae* Koch. Research Journal of Pharmaceutical, Biological and Chemical Sciences vol. 5, issue (4), pp 140-148.

[2] Tehri, K., Singh, N. (2015). The role of botanicals as green pesticides in integrated mosquito management – A review. International Journal of Mosquito Research vol. 2, issue (1), pp 18-23.

[3] Koul, O., Walial, S., Dhaliwal, G. S., (2008). Essential Oils as Green Pesticides: Potential and Constraints. Biopestic. Int. vol. 4, issue (1), pp 63–84.

[4] Kady, M. M., Brimer, L., Furu, P., Lemmich, E., Nielsen, M. H., Thiilborg, S. T., Thastrup, O., Christensen, B. S. (1992). The Molluscicidal Activity of Coumarins from *Ethulia conyzoides* and of Dicumarol. Planta Med. vol. 58, pp 334-337.

[5] El-Bassuony, A. A. (2009). Antibacterial Activity of Two New Monoterpene Coumarins from *Ethulia Conyzoides*. Journal of Pharmacy Research vol. 2, issue (4), pp 582-584.

[6] Aliyu, A. B., Ibrahim, M. A., Ibrahim, H., Musa, A. M., Lawal, A. Y., Oshanimi, J. A., Usman, M., Abdulkadir, I. E., Oyewale, A. O., Amupitan, J. O. (2012). Free radical scavenging and total antioxidant capacity of methanol extract of *Ethulia conyzoides* growing in Nigeria. Romanian Biotechnological Letters vol. 17, issue (4), pp 7458- 7465.

[7] Noumia, E., Houngue, F., Lontsi, D. (1999). Traditional medicines in primary health care: plants used for the treatment of hypertension in Bafia, Cameroon. Fitoterapia vol. 70, pp 134-139.

[8] Baldaa, S. I.; Halim, A. F.; Halaweish, F. T. and Bohlmann, F. (1980). New 5- methylcoumarin Derivatives from *Ethulia conyzoides*, Phytochemistry, vol. 19, pp 1519-1522.

[9] Mahmoud, A. A., Ahmed, A. A., Ilnuma, M., Tanaka, T. (1998). Further Monoterpene 5-methylcoumarins and an Acetophenone Derivative from *Ethulia conyzoides*. Phytochemistry vol. 48 issue (3), pp 543 546.

[10] Shukla, V. S., Dutta, S. C., Baruah, R. N., Sharma, R. P., Thyagarajan, G., Herz, W., Kumar, N., Watanabe, K., Blount, J. F. (1982). New 5-methylcoumarins from *Ethulia conyzoides*. Phyrochemistry vol. 21 issue (7), pp 1725-1731.

[11] Bohlmann, F., Balbaa, S., halim, A., Halawelsh, F. A., (1981). Terpene-Coumarin Derivative from *Ethulia conyzoides*. Phytochemistry vol. 20, pp 177.

[12] Mahmoud, A. A., Ahmed, A. A., linuma, M., Tanaka, T., Muraoka, O. (1994). Structure of a Novel Spiro-monoterpene-coumarin in *Ethulia conyzoides*. Tetrahedron Letters vol. 35 issue (35), pp 6517-6520.

[13] Mahmoud, Z. F., Sarg, T. M., Amer, M. E., Khafagy, S. M. (1983). Anthelmintic coumarin from *Ethulia conyzoides* var. gracilis Asch. & Schweinf. Pharmazie vol. 38 issue (7), pp 486-487.

[14] Balbaa, S. I., Halim, A. F., Halaweish, F. T. (1981). Constituents of the Aerial Parts of *Ethulia conyzoides*. Fitoterapia vol. 52 issue (2), pp 75-76.

[15] Mahmoud, Z. F., Sarg, T. M., Amer, M. E., Khafagy, S. M., Bohlmann, F. (1980). A 5-methylcoumarin Glucoside from *Ethulia conyzoides*. Phytochemistry vol. 19 pp 2029-2030.

[16] Gazal, S.A., Abuzarqa, M., Mahasneh, A.M. (1992). Phytother. Res. vol. 6, pp 265-269.

[17] Abbott, W.S. (1925). J Econ Entomol. vol. 18, pp 625-627.

[18] Finney, D.J. (1971). Probit Analysis. A Statistical treatment of the sigmoid Response curve. 7th Ed .Cambridge Univ. Press, Cambridge, England.

[19] Sun, Y.P. (1950). J Econ Entomol. vol. 43, pp 45-53.

[20] Jamal, A.K., Yaacob, W.A., Din, L. B. A. (2008). Chemical Study on Phyllanthus reticulatus. Journal of Physical Science vol. 19 issue (2), pp 45–50.

[21] Chuan-Rui, Z., Aldosari, S. A., Vidyasagar, P. S. P. V., Nair, K. M., Nair, M. G., (2013). Antioxidant and Anti-inflammatory Assays Confirm Bioactive Compounds in Ajwa Date Fruit, J. Agric. Food Chem. vol. 61, pp 5834−5840.

[22] Isaev, I. M., Agzamova, M. A., Isaev, M. I. (2011). Genkwanin and iridoid glycosides from *Leonurus turkestanicus*. Chemistry of Natural Compounds vol. 47, issue (1), pp 132-134.