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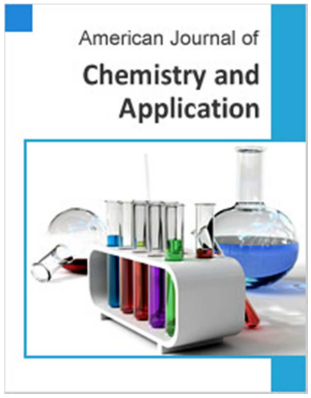

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In vitro Assessment of Pentacyclic Triterpenoids from Leaves of *Pittosporum viridifloruma* Gainst East Coast Fever Vector *Rhipicephalus appendiculatus*

Christine T. Nyabayo[1, *], Josphat C. Matasyoh[1], Charles Mwendia[2]

[1]Department of Chemistry, Egerton University, Egerton, Kenya
[2]Department of Biochemistry, Egerton University, Egerton, Kenya

Email address
nyabayochristine@yahoo.com (C. T. Nyabayo)

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Abstract
Three pentacyclic triterpenoids; oleanolic acid 1, 3, 22, 28- trihydroxyolean-12-ene 2, β-Amyrin acetate 3were isolated from the plant *Pittosporum viridiflorum* (pittosporaceae), and the *in vitro* larvicidal activities of these compounds against East Coast Fever vector *Rhipicephalus appendiculatus* investigated. The mortalities of the larvae were determined after 72 h post treatment. The finding demonstrated that compound 2 was the most pronounced on the larvae with $LC_{50/90}$ values of 53.0(49.75-56.64)/81.84(74.23-74.39) mg/mL followed by compound 3 with $LC_{50/90}$ values of 56.22(52.60-60.45)/90.51(80.63-108.00) mg/mL and finally, compound 1 was the least pronounced on the larvae with $LC_{50/90}$ values of 64.03(59.41-70.37)/106.42(91.67-136.24) mg/mL at 72 h respectively. The results collected bring to limelight alternative sources of more selective, biodegradable and natural larvicidal compounds which can be integrated into new acaricide formulations.

1. Introduction

Ticks have become a primary concern in East Africa since they are vectors of several disease-causing pathogens afflicting livestock [1]. It is also known as the brown ear tick as it majorly feeds on the ear of its hosts [2].The tick *R. appendiculatus* is amongst the principle vectors of *Theileria parva* a causative agent of East Coast Fever [3]. The use of synthetic acaricides against these acari has faced a lot of shortcomings including continuous growing resistance to these acaricides, residues in food, environmental pollution and other effects on non-target organisms. Henceforth there is a need to focus our attention towards medicinal plants extracts from tropical and subtropical regions especially in Africa that are environmental friendly and biodegradable in nature [4].Traditionally, the communities have been using some plants to curb the problems brought about by this ticks. Among the mentioned occasionally used plants included the *Pittosporum viridiflorum* also known as the Cheesewood in English although little research has been done on this plant's pesticidal or acaricidal activity. Previously, infusion of *P. viridiflorum's* leaves has been used to treat cryptococcal meningitis in the Eastern Cape Province of South Africa [5].

According to previous literature, the *P. viridiflorum* plant has been found to be rich in Pentacyclic triterpenoids (PCTs). PCTs are widely disseminated in the kingdom plantae. They are a class of C-30 isoprenoid compounds known to possess some biological effects [6]. The pharmacological relevance of PCTs has increased during the last two decades. They have demonstrated multi-target properties including; wound healing, anti-inflammatory, anti-bacterial, antiviral, hepatoprotective, anti-tumoral effects and low toxicity [7].

2. Experimental

2.1. Plant Material

Fresh leaves of *P. viridiflorum* were collected in August 2013 from Kakamega rain forest (0^0 10'-0^0 21' N 34^0 58' E) in Kenya. A taxonomist identified the leaves at the Department of Biological Sciences, Egerton University. They were placed in polyethene bags and quickly transported to the laboratory to avoid metabolic transformations. *P. viridiflorum* materials were then taken to the center for Herbal Research in Egerton University where they were dried under shade to a constant weight for three weeks.

2.2. Extraction and Isolation of Active Acaricidal Principles

The total acquired 1Kg of the air-dried powder of *P. viridiflorum* was soaked in 5 liters of methanol. Extraction of the methanol crude was done exhaustively at room temperature and concentrated in vacuo at 40^0C.The concentrated crude methanol extract was divided into two portions where one portion was suspended in distilled water to remove carbohydrates and other water soluble substances. Sequential liquid-liquid partitioning was performed starting with hexane followed by ethyl acetate. Both hexane and ethyl acetate extracts were also concentrated to dryness under reduced pressure using rotary evaporator to afford 37.0g and 65.4g of hexane and ethyl acetate crude extracts respectively.

Thin layer chromatography was carried out on the ethyl acetate crude extract to determine the profile and the perfect solvent system for separation. A mixture of EtOAc: $CHCl_3$ at a ratio of 2:3 was selected as the best system since it had the right profile. Column chromatography was then performed using the determined solvent system yielding four fractions (from PF_1 toPF_4). A preliminary screening of the four fraction lead to the selection of PF_2 and $PF_3$for further purification over silica gel preparative TLC. Fractions PF_2 yielded oleanolic acid 1(37mg) and3, 22, 28-Trihydroxyolean-12-ene 2(105mg) while PF_3 afforded β-Amyrin acetate 3 (71mg).

The NMR spectra were recorded on the Burker Advance 500MHz NMR spectrometer at the Technical University of Berlin, Germany. Readings were done in deuterated chloroform ($CDCl_3$) while the chemical shifts of the peaks were assigned by comparison with the residue proton and carbon resonance of the solvent. The internal standard used was tetramethylsilane (TMS) and the chemical shifts were measured in δ (ppm). Elucidation of the structures was done using ACD NMR manager program to get the chemical shift of both carbon and proton atoms. Recording of the mass spectra of the compounds was done on Finnigan Triple Stage Quadrupol Spectrometer (TSQ-70) with electron spray ionization (ESI) method. Analysis of the mass chromatograms was achieved by using the XcaliburQual computer software.

The compounds' mass spectra were recorded on Finnigan Tripple Stage Quadrupol Spectrometer (TSQ-70) with electron spray ionization (ESI) method. Thermo XcaliburQual computer software was used in the analysis of the mass chromatograms.

2.3. Structure Elucidation

The isolated PCTs showed strong fluorescence both under UV (254-365nm) on silica gel TLC plates. Compound1was obtained as a white amorphous powder with anRf value of 0.72 [8]. It was found to have pseudomolecular formula $C_{30}H_{48}O_3$. The positive high resolution electron impact mass spectrometry (HREIMS) of this compound at 16.97 minutes retention time showed ion peaks at *m/z* 457, 479 and 495 that corresponded to $[C_{30}H_{48}O_3+H]^+$, $[C_{30}H_{48}O_3+Na]^+$ and $[C_{30}H_{48}O_3+K]^+$. The ^{13}C NMR showed a total of 30 carbon atom signals; of which two of them resonating at $\delta_{C\text{-}12}$-122.6 and $\delta_{C\text{-}13}$-143.6 were as a result of a pair of sp^2 hybridized carbons [9]. A hydroxyl methine resonating at $\delta_{C\text{-}3}$-79.1and a carboxylic carbon at $\delta_{C\text{-}28}$-182.7 were observed [8].The most downfield signal resonated at δ_C182.7was attributed to the carboxylic carbon (C-28). DEPT NMR spectrum showed the presence of five methine (CH) carbon atoms signals at δ-79.1, 55.2, 47.6, 122.6 and 41.1. A total of 10 methylene (CH_2) carbon atoms signals at δ- 38.4, 27.1, 18.3, 32.6, 22.9, 27.7, 23.4, 45.9, 33.8 and 32.4 were identified. Seven methyl (CH_3) carbon atoms signals were identified at δ- 28.1, 15.6, 15.3, 17.1, 25.9, 33.3, and 23.6. The other missing eight signals were found to be quaternary carbons. The combined spectra data analysis using ^{1}H-, ^{13}C-NMR, DEPT, COSY, and HSQC shows that olean-12-ene type compound is a pentacyclic triterpene [10], [11]. By comparison of these spectral data (Table 1) and that in the literature [9] led to the conclusion that compound 1 is Oleanolic acid and reported here for the first time from *P. viridiflorum*.

The ^{13}C-NMR spectrum of compound2 (Table 1) showed thirty signals, consisting of seven quaternary carbons, six methines, ten methylenes and seven methyls deduced from the DEPT experiments [12]. This gave molecular formulae of $C_{30}H_{50}O_3$. Three methine protons at δ_H-3.0, m, J_1=4.88 Hz, 3.8, d, and 3.5/3.2 dd, J_1=3.85Hz, J_2=3.60Hz showed that the compound had carbon atoms C-3, C-22 and C-28 hydroxylated. The appearance of signals at $\delta_{C\text{-}12}$122.0and $_{C\text{-}13}$144.3 indicated the presence of a double bond in olean-12-ene triterpenoid. ^{1}H-, ^{13}C-NMR, DEPT, COSY and HSQC spectral data shows that compound C is a pentacyclic triterpene [11]. This was further confirmed by the negative HREIMS of this compound at 11.14 minutes retention time showed an ion peaks at *m/z* 457.37 that corresponded to $[C_{30}H_{50}O_3\text{-}H]^-$ By comparison of this spectral data and that in literature [13] led to the conclusion that

compound 2 is 3, 22, 28-Trihydroxyolean-12-ene reported here for the first time from *P. viridiflorum*.

Compound 3 was isolated as a white solid and was found to have molecular formulae $C_{32}H_{52}O_2$ with an Rf value of 0.43. The positive HREIMS of this compound at 16.97 minutes retention time showed an ion peaks at *m/z* 507.33, 491.30, and 469.33 corresponding to $[C_{32}H_{52}O_2+K]^+$, $[C_{32}H_{52}O_2+Na]^+$and to $[C_{32}H_{52}O_2+H]^+$.The ^{13}C NMR spectrum revealed a thirty two carbon atom compound. From the DEPT NMR, spectrum the substituent had two carbons, one quaternary acetoxycarbon ($C_{1'}$-δ_C170.1) and one methyl carbon ($C_{2'}$-δ_C21.3).The proton-proton COSY showed correlations between protons H_2 and H_3 absorbing at δ_H1.44 and δ_H 3.00, H_{11} and H_{12} absorbing at δ_H1.80 and δ_H 5.17 and H_{18} and H_{19} absorbing at δ_H 2.76 and δ_H 1.60 respectively. These correlations helped in the confirmation of the acetate functional group at carbon C_3. ^1H-, ^{13}C-NMR, DEPT, COSY and HSQC spectral data of this compared favorably with that of compound 1 and 2. The comparison of this spectral data (Table 1) and that in literature led to the conclusion that compound 3 is β- Amyrin acetate reported here for the first time from *P. viridiflorum*.

2.4. Larvae Rearing

The larvae of *R. appendiculatus* were reared according to the method adopted from [14] with slight modifications.

2.5. Larvicidal Assay

The bioassays were performed in the Center for Herbal Research at Egerton University. 10-day old larvae were collected from ICIPE and were subjected to contact toxicity according to [15] assay using the *P. viridiflorum* extracts. Larval mortality data was collected after 72 hrs post treatment.

Serial dilution done on compound 1, 2 and 3 stock solutions resulted in 15 concentrations ranging from 80 mg/ml to 10 mg/mL. The concentrations were sprinkled using a pasture pipette on the petri dishes that had whatman No. 1 filter paper (15 cm) attached to the bottom using double sided cellophane tape, and containing 20 larvae each. It was ensured that all the larvae were exposed to the solutions during sprinkling. The experiment was replicated thrice and petri dishes held at 75 % relative humidity at 25°C.The larvae were considered dead if they could not move their appendages when prodded with a pin or exhibit their normal behavior when breathed upon. A set of two 20 larvae was also treated with a positive and a negative control which consisting of 0.2% v/v of Amitraz® and 2% DMSO + distilled water respectively. IBM SPSS 20.0 software was used to calculate the LC_{50} and LC_{90} using log probit regression analysis for the data collected at 95% confidence level.

3. Discussion

The compounds 1, 2 and 3 were confirmed to be PCTs; Oleanolic acids, 3, 22, 28-Trihydroxyolean-12-ene and β-Amyrin acetate respectively. These compounds were confirmed to be actual compounds (As shown in Figure 1 and Table 1) by comparison of their spectroscopic data and their mass spectrometric data with that from literature. Oleanolic acid has also been isolated from *Eriope blanchetti* [16] and *Saturejamutica* [8]. β-amyrin acetate has been previously isolated from *Euphorbia maculate* [17], *Ipomoea pes-caprae* [18]and *Cryptocarya moschata* [18]. These compounds have also been isolated from other plants families. Generally PCTs have been isolated and tested for their insecticidal and acaricidal activities. The compounds α-Amyrin and β-Amyrin are commonly found in medicinal plants leaves species in the *Burseraceae* family such as *Bursera* or *Protium*. Both *in vitro* and *in vivo* studies have shown that β-amyrin acetate also has important biological functions. β-amyrin acetate derivatives proved to be the most potent [19]. α-Amyrin acetate have been extracted from the stem bark of *Ficus palmata* [20], 3, 22, 28-Trihydroxyolean-12-ene structure has been reviewed by [21],[11],[22].

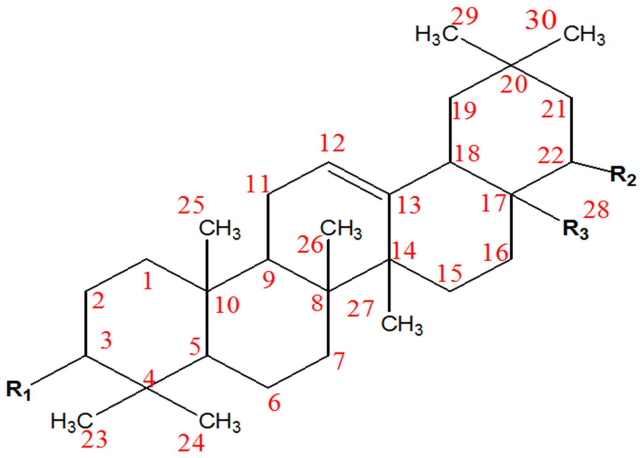
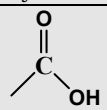
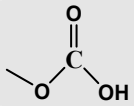


Figure 1. *A skeleton of PCTs.*

Table 1. *Substituents represented by R values for the three PCTs.*

	R_1	R_2	R_3
1	OH	H	
2	OH	OH	OH
3		H	H

PCTs are produced biosynthetically by the transformation of squalene leading to the dammarenyl ring system [23]. Biosynthetically, dammarenyl undergoes ring expansion and additional cyclization to form the characteristic fifth ring found in lupeol, α-amyrin, and β-amyrin skeletons. Oleanolic acid contains the β-Amyrin skeleton. Review of scientific literature dealing with triterpenoids' isolation and identification from natural sources has been reported [24].

No previous studies in literature indicating the evaluation of acaricidal properties of this pentacyclic olean-12-ene type triterpenoids1-3 against *R. appendiculatus* larvae has been found. In the present paper, these triterpenes isolated from the

leaves of *P. viridiflorum* induced lethal effects against the larvae of *R. appendiculatus*. The percentage mortality, LC_{50} and LC_{90} values after 72 h of exposure for the PCTs are summarized in table1, 2 and 3.The finding demonstrated that compound 2 was the most pronounced on the larvae with $LC_{50/90}$ values of 53.0(49.75-56.64)/81.84(74.23-74.39) mg/mL followed by compound 3 with $LC_{50/90}$ values of 56.22(52.60-60.45)/90.51(80.63-108.00) mg/mL and finally, compound 1 was the least pronounced on the larvae with $LC_{50/90}$ values of 64.03(59.41-70.37)/106.42(91.67-136.24) mg/mL at 72 h respectively. The difference can be explained by the difference in polarity of these purified compounds. Compound 2 is the most polar due to the three substituted hydroxyl groups. Polarity affects a compound's physical properties by giving a compound its cohesion and adhesion characteristics. A polar compound can stick itself to the cells of the ectoparasites due to the hydrogen bonds caused by its polarity [25]. The bonds formed are weak [26] and the slightly positive hydrogen atoms formed with the electronegative oxygen atoms in the cytoplasm of the cell, making the compound more cohesive. This factor enhances the potency of the molecule [26]. Studies on the structure–activity on terpenes advocate that molecules with free alcoholic, phenolic or potential olefinic modifications had the best acaricidal activity [27], [28]. The observation [27] that alcohols are the best acaricides against ticks, and that hydrocarbons are least effective, is consistent with the toxicity results obtained against this isolated compounds.

The larvae mortality of the compounds had a dose-dependent effect that is; the activity was proportional to concentration. However, other few studies on acaricidal isolated pure compounds against different stages of *R. appendiculatus* have been reported. PCTs both synthetic and from natural sources have shown to be excellent acaricides [29]. For example, Oleanolic acid has also been isolated from *Eriope blanchetti* [16] and *Satureja mutica* [8]. Oleanolic acid and its derivatives have been reported in non-berry fruits including apple (*Malus pumila*).

Table 2. *^{1}H-NMR (400 MHz, in $CDCl_3$) and ^{13}C-NMR (100 MHz in $CDCl_3$) spectral data of compounds 1-3.*

Carbon	**1**		**2**		**3**	
	^{13}C(δ)	^{1}H(δ)	^{13}C(δ)	^{1}H(δ)	^{13}C(δ)	^{1}H(δ)
1	38.4	1.59(t)	38.7	1.47(t)	39.5	1.49(t)
2	27.1	1.58(m)	27.4	1.46,1.48(m)	27.7	1.44(m)
3	79.1	3.23(t)	77.3	3.00(t)	80.5	3.15(t)
4	38.7	-	38.9	-	39.4	-
5	55.2	3.95(t)	55.2	0.65,0.69(t)	55.3	0.69(t)
6	18.3	1.83(m)	18.5	1.35,1.53(m)	18.5	1.49(m)
7	32.6	0.57(t)	32.6	1.25,1.50(t)	33.9	0.87(t)
8	39.3	-	40.5	-	38.6	-
9	47.6	1.15(t)	47.5	1.5(t)	47.5	1.49(t)
10	37.1	-	36.9	-	35.1	-
11	22.9	1.60(t)	23.5	1.81(t)	23.1	1.80(t)
12	122.6	5.28(t)	122.3	5.12J=3.80Hz(t)	122.0	5.17(t)
13	143.7	-	143.9	-	145.0	-
14	41.6	-	41.3	-	41.8	-
15	27.7	0.95(t)	25.2	0.87(t)	28.4	1.80(t)
16	23.4	0.91(t)	15.6	0.90(t)	27.4	0.99(t)
17	46.5	-	42.0	-	32.8	-
18	41.1	2.81(t)	41.9	2.0(t)	58.9	2.76,dd, J=3.56(t)
19	45.9	1.13(d)	46.1	1.72,1.69(d)	40.0	1.60,1.05(d)
20	30.7	-	31.5	-	40.9	-
21	33.8	2.34(t)	42.7	1.23(d)	31.3	0.87,1.41(t)
22	32.4	1.43(t)	71.4	3.8(t)	41.8	0.87(t)
23	28.1	1.64(s)	28.7	0.93(s)	29.7	0.93,0.86(s)
24	15.6	0.78(s)	16.5	0.87,0.89(s)	15.5	0.64,0.70(s)
25	15.3	0.92(s)	15.8	0.83,0.90(s)	15.7	0.85(s)
26	17.1	1.57(s)	16.9	0.68(s)	16.3	1.50(s)
27	25.9	1.18(s)	26.4	1.12(s)	23.2	1.14,1.10(s)
28	182.7	-	66.9	3.5J=3.85Hz(s)	28.5	0.68(s)
29	33.1	0.86(s)	33.7	0.85(s)	17.5	0.87(s)
30	23.6	0.88(s)	25.1	0.86(s)	21.4	0.87(s)
-	-	-	-	-	170.4	-
oAc	-	-	-	-	21.3	1.93(s)

Table 3. *Larvicidal activity of compounds 1, 2 and 3.*

Concentration in mg/mL	%mortality ± SE		
	1	**2**	**3**
80	86.67±7.64	88.33±2.89	78.33±5.77
75	61.67±7.64	83.33±5.77	75.00±5.00
70	50.00±2.89	85.00±5.00	70.00±2.89
65	46.00±2.89	78.33±10.41	65.00±8.67
60	40.00±5.00	61.67±7.64	58.33±10.41
55	31.67±7.64	50.00±5.00	53.33±10.41
50	25.00±0.00	38.33±2.89	40.00±5.00
45	23.33±10.41	36.67±7.64	33.33±2.89
40	11.67±10.41	20.00±5.00	16.67±2.89
35	10.00±5.00	13.33±5.77	11.67±7.64
30	3.33±2.89	5.00±5.00	1.67±2.89
25	0.00±0.00	0.00±0.00	0.00±0.00
Amitraz®(0.2%v/v)[x]	100.00±0.00	100.00±0.00	100.00±0.00
H_20+2%DMSO[y]	0.00±0.00	0.00±0.00	0.00±0.00
LC_{50}	64.03(59.41-70.37)	53.09(49.75-56.64)	56.22(52.50-60.45)
LC_{90}	106.42(91.67-136.24)	81.84(74.23-94.39)	90.51(80.63-108.00)

[x]Positive control, [y]Negative control

4. Conclusion

Our results have ascertained that the PCTs 1-3 from *P. viridiflorum* have acaricidal actions against the larvae of *R. appendiculatus* However, their effectiveness as acaricides are lower than Amitraz®, a commonly known commercial acaricide. The work supports the ethnobotanical use of *P. viridiflorum* extract as alternative acaricide; that are safer, cheaper, and environmentally benign.

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References

[1] Mathison, B. A., & Pritt, B. S. (2014). Laboratory identification of arthropod ectoparasites. *Clinical Microbiology Reviews*: 27, 48-67.

[2] Wesonga, F. D., Gachohi, J. M., Kitala, P. M., Gathuma, J. M., & Njenga, M. J. (2015). *Theileria parva* infection zero prevalence and associated risk factors in cattle in Machakos County, Kenya. *Tropical animal health and production*, 47: 93-101.

[3] Odongo, D. O., Ueti, M. W., Mwaura, S. N., Knowles, D. P., Bishop, R. P., &Scoles, G. A. (2009). Quantification of *Theileria parva* in *Rhipicephalus appendiculatus* (Acari: Ixodidae) confirms differences in infection between selected tick strains. *Journal of medical Entomology*, *46*: 888-894.

[4] Habeeb, S. M. (2010). Ethno-veterinary and medical knowledge of plant extracts and its method of application (Traditional and Modern) for tick control. *Journal of World Applied Sciences,* 11: 1047-1054.

[5] Otange, M. W, Grierson, D. S and Ndip N. R. (2012). Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. *BMC Compl Altern Med.*12; 1-7

[6] Hussain, H., Al-Harrasi, A., Al-Rawahi, A., Green, I. R., & Gibbons, S. (2014). Fruitful Decade for Antileishmanial Compounds from 2002 to Late 2011. *Chemical Reviews*, 114:10369-10428.

[7] Jäger, S., Laszczyk, M.N., & Scheffler, A. (2008). A preliminary pharmacokinetic study of betulin, the main pentacyclic triterpene from extract of outer bark of birch (*Betulaealba cortex*). *Molecules*: *13*, 3224-3235.

[8] Gohari, A. R., Saeidnia, S., Hadjiakhoondi, A., Abdoullahi, M., & Nezafati, M. (2009). Isolation and Quantificative Analysis of Oleanolic Acid from *Saturejamutica* Fisch. & C. A. Mey. *Journal of Medicinal Plants,* 8: 65-69.

[9] Onoja, E., &Ndukwe, I. G., 2013. Isolation of oleanolic acid from chloroform extract of *Borreria stachydea* [(DC) Hutch. and Dalziel]. *Journal of Natural Products and Plant Resource:* 3:57-60.

[10] Moghaddam, F. M, Farimani, M. M, Salahvarzi, S, Amin, G. (2007). Chemical Constituents of Dichloromethane Extract of Cultivated *Satureja khuzistanica*. *Evidence Based Alternative Medicine:* 4; 95-98.

[11] Mahato, S. B.,& Kundu, A. P. (1994).^{13}C NMR Spectra of Pentacyclic Triterpenoids – A Compilation and some Salient Features. *Phytochemistry;* 37, 1517-1575.

[12] Katti, D. (2014). "Multiscale Modeling and Experiments for Bridging Molecular Scale Interactions to Macroscopic Properties", by Dr. Dinesh Katti Professor & Chair, Dept. of Civil and Environmental Engineering, North Dakota State University.

[13] Seo, Y., Berger, J. M., Hoch, J., Neddermann, K. M., Bursuker, I., Mamber, S. W., & Kingston, D. G. I. (2002). A new triterpenoid saponin from *Pittosporum viridiflorum* from the Madagascar rainforest. *Journal of Natural Products,* 65: 65-68.

[14] Bailey, K. P. (1960). Note of the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. Bulletin of epizootic diseases of Africa, 8: 33-43.

[15] FAO. FAO Animal Production and Health Division, Rome; 2004.

[16] Silva-Aguayo, G. (2006). Botanical Insecticides. Radcliffe's IPM World Textbook. University of Minnesota. Available atwww.radcliffes/ipm/network.htm.

[17] Matsunaga, S., Tanaka, R., & Akagi, M. (1988). Triterpenoids from *Euphorbia maculate*. *Phytochemistry*: 27535-537.

[18] Schinor, E. C., Salvador, M. J., Turatti, I. C., Zucchi, O. L., & Dias, D. A. (2004). Comparison of classical and ultrasound-assisted extractions of steroids and triterpenoids from three *Chresta* spp. *Ultrasonics Sonochemistry*, 11: 415-421.

[19] Johann, S., Soldi, C., Lyon, J. P., Pizzolatti, M. G., &Resende, M. A. (2007). Antifungal activity of the amyrin derivatives and in vitro inhibition of *Candida albicans* adhesion to human epithelial cells. *Letters in applied microbiology*: 45, 148-153.

[20] Sirisha, N., Sreenivasulu, M., Sangeeta, K., & Chetty, C. M. (2010). Antioxidant properties of Ficus species–A Review. *International Journal of Pharmtechnological Research*, *2*(4), 2174-2182.

[21] Tori, K., Seo, S., Shimaoka, A. &Tomita, Y. (1974) *Tetrahedron Letters* 4227.

[22] Dinda, B., Debnath, S., Mohanta, B. C., &Harigaya, Y. (2010). Naturally occurring triterpenoid saponins. *Chemistry & biodiversity*, 7: 2327-2580.

[23] Habila, J. D., Shode, F. O., & Opoku, A. R. (2011). Triterpenoids from *Eucalyptus grandis* Hill ex Maiden inhibits platelet aggregation. *African Journal of Microbiology Research*, 5: 4646-4651.

[24] Moreau, R. A., Whitaker, B. D., Hicks, K. B. (2002). Phytosterols, phytostols and their conjugates in foods: structure diversity, quantitative analysis, and walth-promoting uses. *Prog Lipid Res*; 41,457-500.

[25] Boucias, D., & Pendland, J. C. (2012).*Principles of insect pathology*. Springer Science & Business Media.

[26] Lukens, R. J. (2013). *Chemistry of fungicidal action*. Springer Science & Business Media.

[27] Perrucci S, Macchioni G, Cioni PL, Flamini G, Morelli I. (1995). Structure/activity relationship of some natural monoterpenes as acaricides against Psoroptes cuniculi. *Journal of Natural Products*, 58: 1261–1264.

[28] Cantrell, C. L., Klun, J. A., Pridgeon, J., Becnel, J., & Duke, S. (2009). Structure/Activity relationship studies on the arthropod repellent callicarpenal. USDA-ARS. Available from: http://www.ars.usda.gov.

[29] Rizental, M. S. (2014). Effect of plant extracts and organic substances on mortality of *Cornitermes cumulans* (Isoptera: Termitidae).