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Physiochemical Characterization and Evaluation of Insecticidal Activities of Delonix Regia Seed oil against Termite (Odontotermes obesus), Ticks (Ixodes scapularis) and Cockroach (Blattella germanica)

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Abstract
Plant produces large number of compounds as secondary metabolites with complex structure and diversity with a potent insecticidal activity. Large number of plants has been identified so far for their insecticidal property and can be used as an alternative pest management for synthetic pesticides. The present study reports the physiochemical characterization and biocide from delonix regia seeds oil. The biocide potential of the seeds oil was evaluated against termite (Odontotermes obesus), tick (Ixodes scapularis) and cockroach (Blattella germanica). The bioassay study showed that Delonix regia 10% oil caused 100% mortality in 32 hrs, 41 hrs and 50 hrs against termite, tick and cockroach, respectively. The LD_{50} was determined to be 0.574%, 0.753% and 1.12% for termite, tick and cockroach, respectively for delonix regia oil after 32 hrs exposure. The biocidal potential of the oil is statistically significant ($p < 0.05$) when compared with blank and solvent controls at all concentration tested. Physicochemical parameters were also evaluated in accordance with American standard testing method specifications.
Keywords: Anti-Termite, Anti-Tick, Anti-Cockroach, Biopesticide, Physicochemical Parameters

1. Introduction
Plant produces large number of compounds as secondary metabolites with complex structure and diversity. Secondary plant metabolites play a key role in plant defense against insects [1]. Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. They classified into three major classes based on the type of active ingredient used, namely microbial, biochemical, or plant incorporated protectants. The active ingredient can be a single molecule or a mixture of molecules, such as a naturally occurring mixture comprising a plant essential oil, or a mixture of very structurally similar molecules called isomers in the case of insect pheromones [2]. Delonix regia (boger) Raf, (Royal Poinciana, flamboyant) is an ornamental flowering tree related to the mimosa tree, family (Leguminosae, subfamily), native and endemic to Madagascar. This species is considering one of the five most beautiful flowering trees in the world, blooming between spring and early summer. The flower is very show and its color varies from orange to orange-red, including yellow and a rare white [2, 3].

The flowers are reputed to produce bee forage while large pods as well as the wood are used for fuel. The tree could provide timber. The bark has medicinal properties while the hard, elongated seeds are occasionally used as beads. Delonix regia (D. regia) is mainly valued as a decorative tree, often being planted in avenues and gardens. It could also be planted as live fence posts (boundary or barrier or support) [4]. Chemical constituents of different classes such as; proteins, flavonoids, tannins, phenolic compounds, glycosides, sterols, and triterpenoids were reported from flowers and leaves of Delonix regia (Hook) Raf species[5]. The decoction of the leaves is traditionally used in treating gastric problems, body pain, and rheumatic pains of joints [6].

The flower of Delonix regia (Hook) Raf was used as natural color, as an acid-base indicator[5], antiarthritic, anti-inflammatory [6, 7] and insecticide [8]. The leaves are reported for its antimicrobial, antihypertensive, antimalarial and antioxidant effect[9, 10]. The bark possesses termiticidal properties [11]. The seeds of delonix regia yield thick and dark green oil for medicinal use, hair grease [12] and useful alternatives source of biodiesel [13]. Oils extracted from plant sources have a rich history of use by local people as a source of food, energy, medicine and for cosmetic applications. It has been used in the production of lubricants, soaps and personal care products, as well as in the topical treatment of various conditions such as hair dandruff, muscle spasms, varicose veins and wounds. In recent years, demand for seed oils as ingredients for food, cosmetics and biofuel has greatly increased as industry seeks natural alternatives[14]. Some of the bioactive components extracted from *delonix regia* have insecticidal property. The aim of the present study is also to evaluate the insecticidal activity of the n-hexane extracts from *delonix regia* seed oil against termite (*Odontotermes obesus*), tick (*Ixodes scapularis*) and cockroach (*Blattella germanica)* and its physiochemical characterization.

2. Materials and Methods

2.1. Sampling and Sample Preparation

The pods of Delonix regia were harvested from Arbaminch University, Institute of Technology in February 2015. The harvested pods were then broken off mechanically to release seeds. Then, the seeds were separated from the chaff, sorted and cleaned to remove all foreign matter such as dust, dirt, immature and broken seeds. The cleaned seeds were air dried at room temperature for a week followed by grinding in a grinding mill. Finally the seed powder was kept in polyethylene bag until further use.

2.2. Oil Extraction

Essential oil was extracted from 30 g of powder; poured in a tumbel and fed to a lab-scale Soxhlet extractor fitted with a 1L round-bottom flask and a condenser. The extraction was carried out using 250mL of n-hexane for 8 hours. Then, the solvent was separated from the oil by using Rota evaporator and suction pump. The experiment was repeated in triplicate. The total extraction process was completed within three hour. The extracted phase was distilled to separate the oil from the solvent. The extracted oil yield was expressed as percentage, which is defined as weight of oil extracted over weight of the sample taken. The Physiochemical parameter like acid value, saponification value, peroxide value, iodine value, cloud point, refractive index, solubility, pH value was determined by using the oil that kept for further analysis.

2.3. Insecticidal Value Delonix regia Seed oil

The three types of insects, termite (*Odontotermes obesus*), tick (*Ixodes scapularis*) and cockroach (*Blattella germanica)* identified for the present study were collected from Arbaminch, Ethiopia. The collected insect were brought to the laboratory and acclimatized for about 24 hours. Each batch of insects, held in a rigid polythene container with a mesh lid, was transferred to the test room maintained. An l0 mL plastic beaker containing cotton wool soaked with l0 mL water was inverted on the mesh to provide a water source for the insects. Hence, each time oil samples of 1, 2.5, 5 and 10 % oil solution in 20 % of ethanol was made and 1mL of the solution was taken in testing Jars/Petri dish. Food or water was given during the test period after every count. After the insects had recovered, knockdown counts will be recorded every 5 hours. Modified bioassay method was employed to evaluate the biocidal activity of *delonix regia* oil.

2.4. Data analysis

The data collected was analyzed statistically using Origin 6.0 and SPSS version 16 (SPSS Inc., Chicago, IL, USA). One-Way Analysis of Variance (ANOVA) followed by Turkey's Honestly Significant Difference (HSD) for mean comparison between values of the treatment will be used. Values of $P < 0.05$ will be considered significant.

3. Results and Discussion

3.1. Oil Extraction and Physicochemical Characterization

The calculated percentage oil/dry weight extracted, using Soxhlet extraction technique and n- hexane from delonix regia seed was 30.8%. The physicochemical parameters were within American Standard Testing Method (ASTM) (Table 1 and 2). The results were compared with Pyrethrin Pesticide.

Table 1: Physical Properties of delonix regia oil vs. Pyrethrin[1]

Parameters	**ASTM Specifications/ Pyrethrin**	**Delonix Regia Oil (This study)**	**Significance**
Boiling point (^{o}C)	192-193	69	Identification
% of seed oil	-----	30.8	Identification
% of moisture content	-----	6.38	Identification
Refractive index	0.5-0.89	1.42	Identification
Color	------	Deep green	identification
Odour	------	odourless	identification
Cloud point (^{o}C)	------	13	
Solubility	Insoluble in water	Insoluble in water Soluble in acetone partially soluble in ethanol	Less leaching to groundwater
Specific Gravity	0.70	0.743	Identification
EC_{50} (32 hrs.) for 10 % of substance (termite)	0.12% (w/w)	0.472% (w/w)	Toxic to termite workers
Surface tension (N/m)	0.060-0.057	0.04	Less risk to surface water
Partitioning coefficient (log K_{ow})	2.71-5.2	1.69	Less Lipophilic
Bioconcentration Factor (BCF)	100-11,000	1.8	Less bioaccumulation
Density (g/ml)	-------	0.621	Higher energy

*ASTM = American Standard Testing Method

Table 2: Chemical Properties of Delonix Regia oil vs. Pyrethrin

Parameters	**ASTM Specifications/ Pyrethrin**	**Delonix Regia Oil (This study)**	**Significance**
pH of 10% solution	3 to 9	6.88	Accidental-oral ingestion
Peroxide Value (Meq/g of oil)	10.79 ± 1.16	11.86	non-combustible
Saponification Value [mg KOH /g of Oil]	90 – 105	22.44	Moderate free fatty Acid (FFA) content
Acid Value (gm NaOH/ 10 gm of Oil)	200-2600 mg/g	1.683 mg NaOH /g	Decomposed by mild acids and alkalis
Iodine Value (g/100g of oil)	----------	23.48 g/ 100 g of oil	Unsaturated = favours biopesticidal potential

Iodine value is a measure of the unsaturation level and the reactivity of the oil. The higher the iodine value, the greater the degree of unsaturation is, thus the more fluid [15]. The iodine value was found to be 23.38 g /100 g oil as shown in Table 2. The value is below 100 and as such the oil can be classified as non-dry oil. This value also represents the decrease in unsaturation of oil, which is beneficial in the sense that the lower the unsaturation of oils and fats, the greater will its oxidation stability be. Saponification value is a measure of the alkali reactive groups in fats and oils and is useful in predicting the type of glycerides in an oil sample. Saponification value is obtained by determining potassium hydroxide in mg required to saponify 1 g of fat. Delonix regia oil has low saponification value of 22.44 mg KOH/g oil as shown in Table 2. Saponification value indicates the average molecular weight of the oil. Measuring saponification value means molecular mass can be obtained. Saponification value is inversely related to mean molecular mass. A higher saponification value indicates that there is a greater portion of low molecular weight fatty acids. High value of acid can indicate the presence of oxidation products in the oil bath; this can cause corrosion and sludge in your system.

Peroxide value is a measure of extent of glycerides constituent decomposition by lipase action, which is added by light, air and moisture. It is also an indication of the level of rancidity of the oil [15]. In the present study peroxide value of delonix regia oil was found to be 11.86 meq g^{-1} and these low peroxide value increases the suitability of the oil for long time storage due to a low level of oxidative and lipolytic activities. A higher density means more mass of oil per unit volume. Delonix regia seed oil has a density of 0.621g cm^{-3}, which is high. The higher mass of oils would give higher energy available for work output per wit volume. The results for Surface tension, Solubility and pH of 10% solution indicates that; this oil has less effect on both the abiotic and biotic components of the environment, while poison against several pests. It was effectively used to combat Termites, Cockroaches, and Ticks population. Therefore, delonix regia seed oil is a suitable Biochemical pesticide; substitute chemical pesticides used in controlling pests.

3.2. Biocidal activity of Delonix regia Oils

Plant metabolizes various compounds as a secondary metabolite with structural diversity and complex nature. Plant synthesizes these chemicals for various purposes where utilization as a protective agent against insects and pathogens can be pointed. In the present study the insecticidal activity of crude extract of *delonix regia* against termite (*Odontotermes obesus),* Tick (*Ixodes scapularis*) and cockroach (*Blattella germanica)* was studied. The percentage mortality of termite, tick and cockroach were calculated after treatment of the insect with the plant extract. The percentage mortality of tick were analyzed and given in Figure 1. As it can be understood from the result, the percentage mortality of tick increased with increase in concentration and time of exposure. The highest mortality for tick was recorded at a concentration of 10 %. At this concentration the tick encounters death within 41 Hrs. The LD_{50} was determined to be 0.753 %. The recorded insecticidal activity of the plant extract showed statistical significance ($p < 0.05$) for each concentration (1 %, 2.5 %, 5 % and 10 %) compared to control.

The percentage mortality of adult termite (*Odontotermes obesus*) exposed to different concentration of plant extract was presented in Figure 2. The percentage mortality of termite increased with increase in concentration and time of exposure. At 10% concentration the termite encounters death within 32 Hrs and the LD_{50} was determined to be 0.574 %. The insecticidal activity of seed extract of *delonix regia* showed statistical significance ($p<0.05$) for each concentration (1 %, 2.5 %, 5 % and 10 %) compared to control. Similarly, the percentage mortality of cockroach were analyzed and given in Figure 3. As it can be understood from the result, the percentage mortality of cockroach increased with increase in concentration and time of exposure. At 10% concentration the cockroach encounters death within 50 Hrs. The LD_{50} was determined to be 1.12 %. The recorded insecticidal activity of the plant extract showed statistical significance ($p < 0.05$) for each concentration (1 %, 2.5 %, 5 % and 10 %) compared to control. The percentage mortality of termite, tick and cockroach at a concentration of 10 % were compared and the result was presented in Figure 4. *Delonix regia* showed better insecticidal activity against termites compared to that of tick and cockroach.

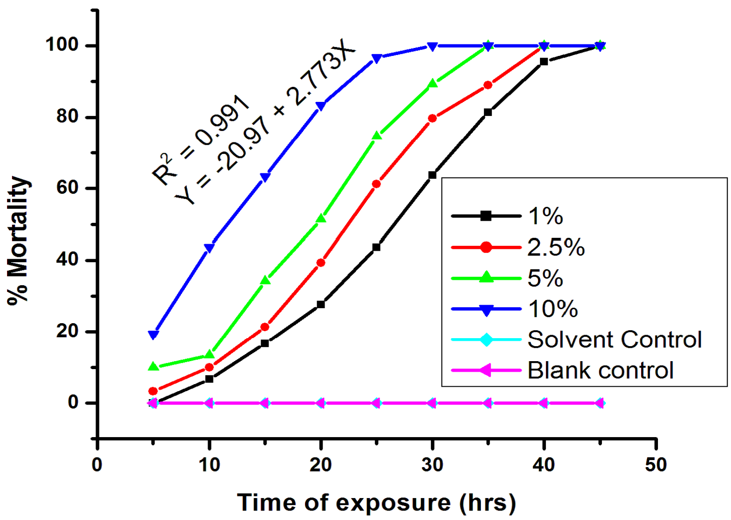

Fig 1: Plot of percentage mortality of Ticks time with different concentration of Delonix regia seed oil

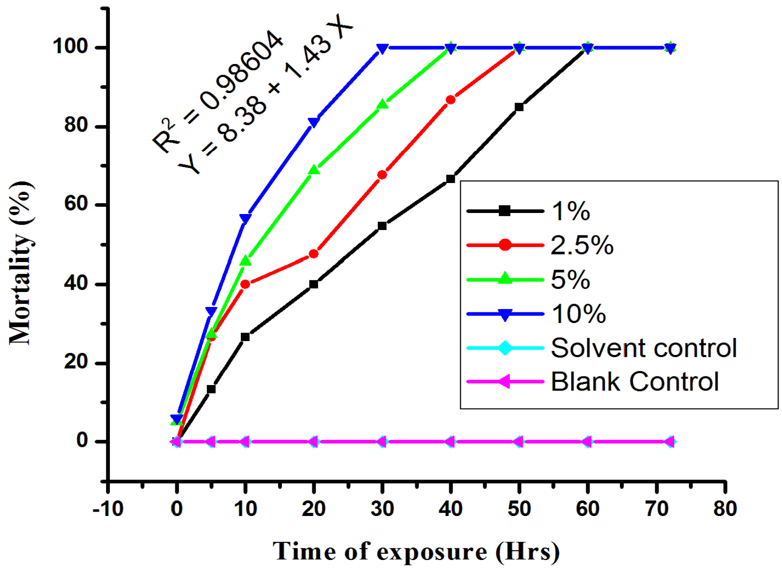

Fig 2: Plot of percentage mortality of Termites time with different concentration of delonix regia seed oil

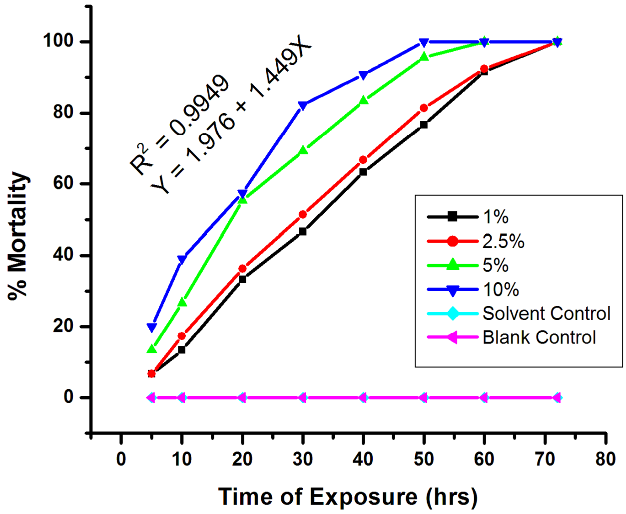

Fig 3: Plot of percentage mortality of Cockroaches time with different concentration of Delonix regia seed oil.

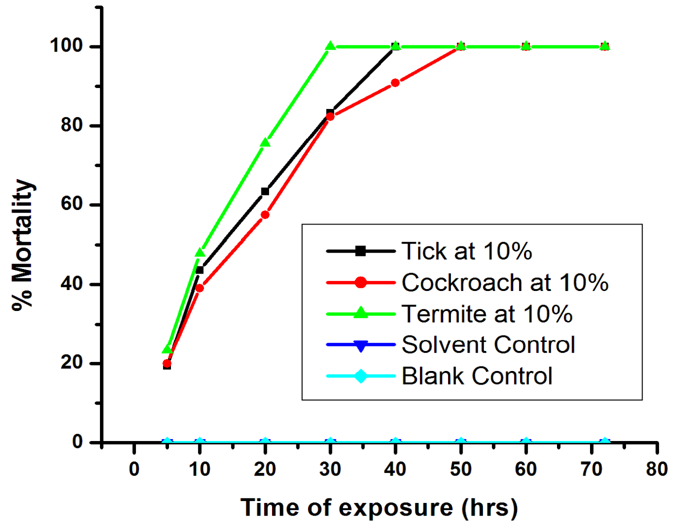

Fig 4: Comparative plot of percentage mortality of cockroach, termite and tick workers vs. time with 10% of Delonix regia seed oil.

3.3 Discussion

A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity. Collectively plants produce remarkably diverse array of over 100,000 low molecular mass natural products, also known as secondary metabolites. Most of these are derived from the isoprenoid, phenylpropanoid and alkaloid or fatty acid pathways [16]. It is quite interesting to know that the delonix regia seeds are known to contain several alkaloids of pharmaceutical importance as already mentioned. Research into plant metabolites for human disease management is many as against pathogens management; it is hence the present study is conducted to demonstrate the possible application of herbal remedy for the plant disease management. The meaningful use of this weed depends on exploitation of its biological activity in agriculture; in view of this the present study is highly successful in indicating its biopesticidal potential against pathogens. The present study has demonstrated, for the first time, the biopesticidal potential of *delonix regia* against *Termite, Tick and Cockroach*.

Ability of the oil rich fraction obtained from seeds of delonix regia to inhibit growth of almost all the tested pesticide species revealed a broad spectrum biopesticidal property of the extracts that oils are used by the plants in defense mechanism against pathogens [17]. The earlier reports of Kandasamy, 1989 [18] have indicated biopesticidal properties of leave extract of delonix regia, but the present investigation shows that seed extract, also have the potential to inhibit pesticide growth. In our study, the growth of *termite, tick and cockroach*, that were found to be resistant towards many standard pesticides, was remarkably inhibited by the oil fraction; this shows the potential of these plant parts to control the growth of drug resistant pathogens. It could also be concluded that the biopesticidal compound extracted from delonix regia may inhibit termite, tick and cockroach by a different mechanism than that of currently used pesticides. In comparison to chemical pesticides, plant derived products have many advantages in insect pest management strategies. In the present study, exposure of termites, ticks and cockroaches to various concentrations of the extracts made in solvent indicated that the toxic effects were greater on termite, tick and cockroach workers at 32hr, 41hr and 50hr test duration respectively. The observed termite, tick and cockroach effects of this no-choice experiment could be because of the presence of different constituents in the extract. A highest mortality rate was recorded (100%, 32 hr, 41hr and 50hr) with 20% ethanol of *delonix regia.* In conclusion, a high mortality was found in the extracts, in which all the secondary metabolites i.e. terpenoids, flavonoids, tannins and alkaloids were present.

4. Conclusion

Seed oils have been used for centuries by rural communities as food, medicine, for cosmetic applications and as fuel. The extraction and characterization of Delonix regia seed oil results show that delonix regia seed oil is a very important raw material for industries. It can be used in industrial production of various products like paints, varnishes, soap, shampoo, lubricants and fertilizers. The percentage oil content is high and this suggests that its extraction on commercial scale is possible and economical. The physicochemical parameter of Delonix regia seed oil indicates this oil can be used in soap and detergent industries. Because oil exhibit low saponification value contains more of unsaturated free fatty acid and this makes the oil good in quality and higher in molecular mass. Thus, oil with less saponification value is good for soap production. In general, delonix regia seed can yield high amount of oil. The seed oil produces different chemicals as secondary metabolites which help them to defend themselves from insects and others organisms. Secondary plant metabolites can also be extracted and used as controlling devise against insects as cockroach and termites. In the present study the n-hexane extract of *delonix regia* showed a significant insecticidal activity against cockroach, tick and termites. Hence from this prospective it might be suggested that the proposed plant can be used as an alternative method to control insects. But further studies would still be required for better understanding of especially the chemistry of delonix regia seed NMR, GC-MS, MS, LC-MS, Elemental analyzer, X-Ray crystallography. The researchers believe that field level studies would be needed to further validate and reproduce biopesticidal potential of this seed oil.

5. References

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