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Full Length Research Paper

Anti-mosquito repellent from Artemisia Annua

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Malaria is one of the dangerous diseases, which are occurred due to mosquitoes. The mosquitoes are increase day by day's industrial growth and awareness. Protection from arthropod bites is best achieved by avoiding infested habitats, wearing protective clothing, and using insect repellent. In many circumstances, applying repellent to the skin may be the only feasible way to protect against insect bites. Given that a single bite from an infected arthropod can result in transmission of disease, it is important to know which repellent products can be relied on to provide predictable and prolonged protection from insect bites. Commercially available insect repellents can be divided into two categories synthetic chemicals and plant derived essential oils. In that extracted oil are more effective, non-sticky; non-toxic and environmentally friendly; safer on sensitive skins and some can be used on children as young reduced irritation; harmless to most plastics and fabrics. The aim of the work is to use extracted oil from Artemisia Annua as anti-mosquitoes repellent. The extracted oil was used with eucalyptus oil, neem oil and rose oil. The better performance shows by *Artemisia Annua* with eucalyptus oil combination. Up to 2 hours not single bite by female's mosquitoes

Keywords: Antibiotics; Biological; Diseases; Eucalyptus; oil; Rose

INTRODUCTION

Malaria history is not a new phenomenon of World. Some of the earliest references to this fever occur in the Atharva Veda believed to have been composed about 1500 B.C. Vndyke Carter and others verified the discovery of malaria parasite by Laveran in 1881 quickly in India, but it is not till later that minds of research workers began to be directed towards the association of the insects with disease (Ali et al., 2004). Malaria (marsh fever, periodic fever) is a parasitic disease that involves infection of the red blood cells (RBCs). Malaria is caused by the protozoan parasites belonging zoologically to the class sporozoa (Murray et al., 2012). These parasites are peculiar to man, who constitutes their intermediately host and in whose red blood corpuscles they live and multiply and may give rise to a periodic fever associated with anemia, enlargement of spleen, and the deposit of black pigment in that organ and elsewhere (Kassaye et al., 2006). The malaria as a communicable disease caused by sporozoan parasites of the genus Plasmodium SP and transmitted to man by certain species of infected female Anopheline mosquitoes. Periodic chills & fever, enlargement of spleen and secondary anemia with a tendency to relapse characterize the disease clinically (Deressa et al., 2000). All mosquitoes must have water in which to complete their life cycle. This water can range in quality from melted snow water to sewage effluent and it can be in any container imaginable. The type of water in which the mosquito larvae is found can be an aid to the identification of which species it may be (Nallan et al., 2006). Also, the adult mosquitoes show a very distinct preference for the types of sources in which to lay their eggs. They lay their eggs in such places such as tree holes that periodically hold water, tide water pools in salt marshes, sewage effluent ponds, irrigated pastures, rain water ponds, etc (Celeghini et al., 2006). Each species therefore has unique environmental requirements for the maintenance of its lifecycle. The life cycle of the mosquito is shown in Figure 1.

Control of mosquitoes is something of utmost importance in the present day with rising number of mosquito borne illnesses. Mosquitoes need to be exterminated using the right tools and with a little bit of effort (Zhenghao et al., 2007). These blood thirsty beasts

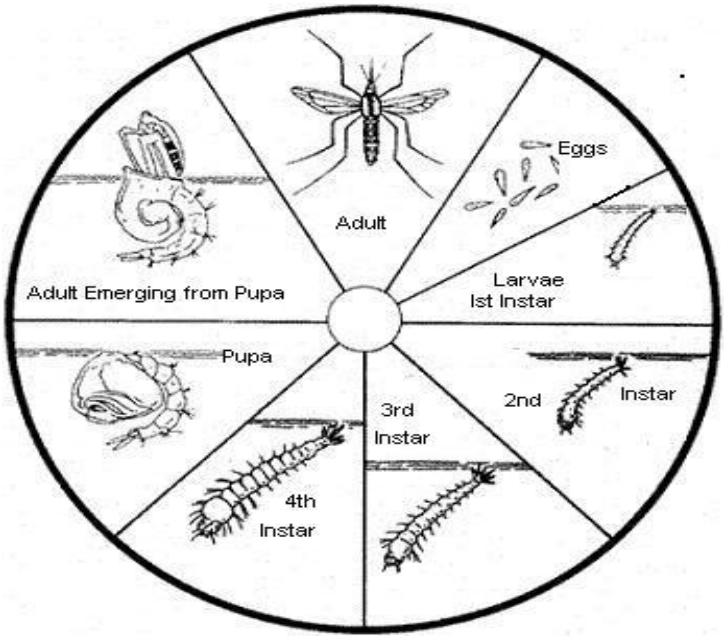

Figure 1. Life cycle of mosquito (arlingtonva)

do not care about boundaries and they can bite you if your neighborhoods are allowing its breeding. So the mosquito control measures can be successful only if public mosquito programs are designed. First and foremost thing is to destroy the breeding areas of these mosquitoes (Tatem et al., 2010). The mosquitoes are horrific -- they're highly aggressive, you can be bitten hundreds of times without protection, its torture, impossible to bear. Deforestation and industrialized farming are also two of the factors causing an alarming increase in the range mosquitoes (Fang et al., 2005).

The World Health Organization says global warming is also expanding the range of mosquitoes that carry malaria, yellow fever, and dengue fever, putting millions more humans at risk. Malaria mosquitoes are appearing in upland areas where they've never been seen before .A child dies of malaria every 12 seconds (WHO 2003). Mostly in the Third World, in the history of the world, more people have died from diseases transmitted by mosquitoes than from all the fighting in all the wars, says appropriate technology company Jade Mountain (WHO, 2008)."The world's most dangerous animal is the mosquito," according to a BBC World Service health program: malaria now infects approximately 110 million people annually, causing 2-3 million deaths, and with increasing drug resistance, the problem is worsening, while attempts to control the mosquitoes with pesticides have proved ineffective (Namdeo et al., 2006; Henderson, 2009). Escaping from mosquito is nowadays a great necessitate in the world as they are posing much irritation and even throwing many to death. They are the carriers of many harmful diseases like West Nile Virus disease; Malaria, Dengue Fever, Chikungunya, Lyme disease etc (White, 2008). Dengue fever and Dengue Hemorrhagic Fever are the most common mosquito-borne viral diseases that affect a wide range of people in the world. It is caused due to the bite of an infected Aedes Mosquito (Snow et al., 2005). It is a threatening disease as there is no proper vaccine or treatment yet found. These diseases are caused only due to our carelessness. Actually we our self is getting in to the danger. The only way to get away from these diseases is by taking proper protection as soon as possible. There are number of things you should take care to avoid being bitten by mosquitoes. Wear long-sleeved shirts and long pants tucked into socks while you are working outdoors. While indoors, stay in air-conditioned or screened areas or use bed nets (Ro et al., 2006). Avoid mosquito breeding by clearing stagnant water from drains. Another important method to break out from mosquitoes is by the usage of Repellent. The main goal of the study is to examine the extracted oil form Artemisia Annua as anti mosquito repellent. The experimental oil was mixed with different herbal oil eucalyptus, neem and rose to test the performance.

MATERIAL AND METHODS

Material

Botanically, *Artemisia annua* is a vigorous weedy annual which is a single-stemmed and ranges in height from

Figure 2. Artemisia annua plant leaves

one to two meters. It grows easily in temperate areas and tropical areas at higher altitudes and is raised in an increasing number of countries. It is well suited to both small-scale and plantation culture. The seed is extremely small and is usually grown to the seedling stage and transplanted (Zhenghao et al., 2007). The best quality seed, in terms of production of leaves and yield of artemisinin, is provided by certain forms of purchased seed, which are generally limited in supply. Relatively few inputs are needed, aside from some fertilization, because the plants at present do not seem to have any particular insect or disease problems (this could change). Normally, some water is required to establish the crop and dry weather is needed at the harvest and for drying. Artemisinin levels of the plants tend to vary by variety, but the influences of area and growing conditions are not yet clear. It is principally planted early in the calendar year and needs five to six months to mature (Worrall et al., 2004). The image of Artemisia is shown in Figure 2.

The leaf of Artemisia annua growth stage of five month was collected before the flowering of the plant, from Wondogenet (270 km south west of Addis Ababa), kombolcha and kalu (360-376 Km in north east of Ethiopia) and Gonder (724 Km north west of Ethioia) where it is cultivated for different research purposes.

Methods

Essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile or ethereal oils or simply "oil off" the plant from which they were extracted. Oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Essential oils do not as a group needs to have any specific chemical properties in common, beyond conveying characteristic fragrances. An essential oil is a liquid that is generally distilled, most frequently by steam, from the leaves, stems, flowers, bark, roots, or other elements of a plant and they contain the true essence of the plant it was derived from. The oils are generally extracted by distillation. Other processes include expression, or solvent extraction. They are used in perfumes, cosmetics, soap and other products, for flavoring food and drink, and for scenting incense and household cleaning products. Extract artemisinin from Artemisia annua by using hexane solvent with soxhlet extractor.

Extraction

The extractor consist a heating device, solvent reservoir and extract flask (round bottom flask), an extraction chamber containing a porous paper extraction thimble and a condenser. 100g of *artemesia annua* be placed in the thimble and covered with glass wool. It is placed in the extraction chamber. The extract flask is weighed and about 1800 mL of solvent poured into it. The system is then heating. After above 3 hours, the solvent and extract in the extract flask be vaporized using a rotavapour. The experimental setup is shown in Figure 3.

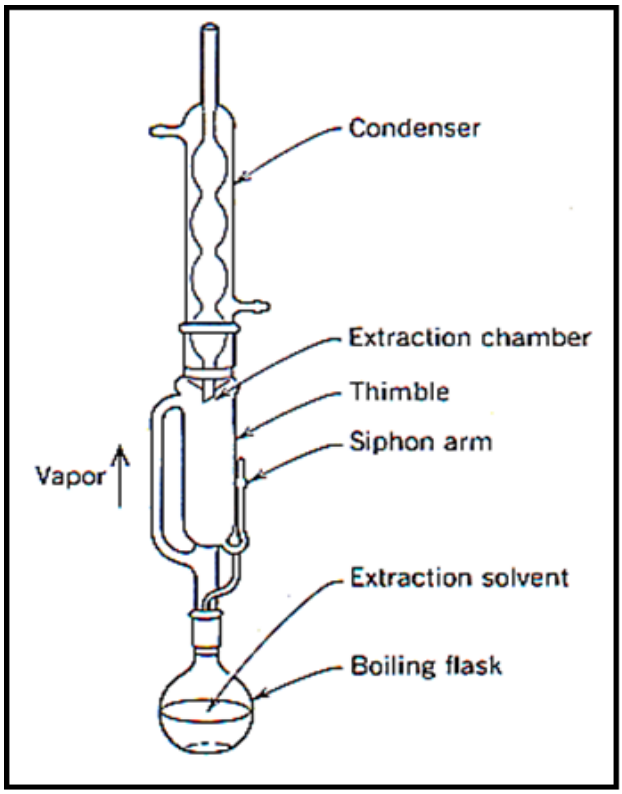

Figure 3. Experimental setup soxhelet extractor apparatus

Experimental steps

1) Weight 100gm of dried Artemisia annua for extraction
2) Put the ground sample in thimble
3) Set up the Soxhlet apparatus
4) Weight the empty and dry round bottom flask of Soxhlet apparatus
5) Fill the thimble with 3/4th of its volume with hexane or ethanol solvent
6) Put the thimble in the middle piece of the Soxhlet extractor
7) Weight the round bottom flask
8) Pour enough amount of hexane or ethanol in the round bottom flask of extractor, which is pre weighted
9) Extract the artemisinin until the solvent become colorless
10) Separate the solvent from artemisinin using rotary evaporator
11) Put the extracted artemisinin in oven for 240 min to remove residual solvent
12) Cool the flask contacting the artemisinin in desiccators
13) Weight the flask with the extract and calculate the % age artemisinin in Artemisia Annua

Analysis

The laboratory work for extraction of artemisin from Artemisia Annua by using hexane as a solvent in Soxhlet apparatus gave 0.8 % artemisinin content. Rotary evaporator recovers almost 75 % of hexane solvent. From literature review and Ethiopian essential oils extraction center laboratory results showed with different places give 0.5 – 1.2% artemisinin content. Therefore by using this laboratory result, search the right kind large extractor for massive capacity.

Anti mosquito test

The repellent trials were conducted in a repellent test chamber (30 x 30 x 62.5 cm) under laboratory conditions. Three concentrations—10, 20 and 30% of the repellents in mustard and coconut oil were evaluated on the hands of human subjects. Before application of

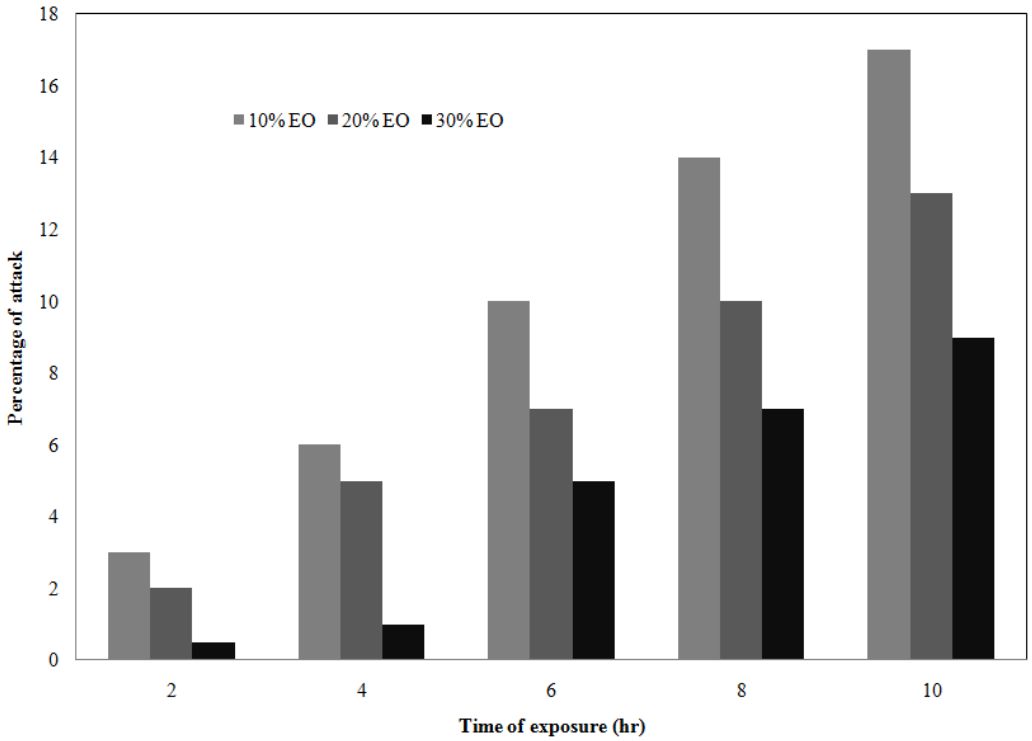

Figure 4. Effect of Artemisia Annua with eucalyptus oil

the repellents, hands were washed and cleaned thoroughly with rectified spirit. Male and female Aedes (S.) albopictus mosquito progenies obtained from

laboratory colony were maintained in honey solution in a cloth cage (50 x 50 x 50 cm) under controlled temperature (28 + 2°C) and relative humidity range (75–80%). About 50 to 60 hungry (3 days old) females Ae. (S.) albopictus were introduced into the repellent chamber through the hole on top. Aliquot of 0.3 ml of the test solution was smeared on dorsal side of one hand (wrist to finger tips) of each of the subject. After 2hours of application, the hand was placed inside the repellent chamber for 30 min through a hole up to wrist and plugged with cotton to prevent escape of mosquitoes in order to facilitate the female mosquitoes to bite on. The test was repeated at every 2hours interval. The interval between the application of repellent and the first two consecutive bites occurring within 2hours was considered as protection time against the bites afforded by each of the concentrations of the test repellents20. The test was replicated 10 times for each concentration of the repellents. Control readings were obtained by placing hand inside the repellent chamber without applying any repellent before the experiment (Mark et al., 2002). Results obtained were statistically analysed as per Randomize Block Design test (RBD).

RESULTS AND DISCUSSION

Efficiency with Artemisia Annua with eucalyptus oil: The effectiveness of Artemisia annua was carried out with EL oil with different percentage of extracted oil from Artemisia annua (AN), which represented in Figure 4. It was found when extracted oil percentage is 30% there is no bite almost up to 2h means 0.5% by *Aedes* (S.) *albopictus* mosquitoes. When the percentage of extracted oil was decrease to 20%, only 2 bites and extracted oil percentage was 10% there is 3 bites by female *Aedes* (S.) *albopictus* mosquitoes. The anti-mosquito repellent efficiency was decrease with increase with time it was effect up to 10h of experiment. The effectiveness was also decrease with decrease in percentage of extracted oil. This may be due to evaporation of extracted oil with the atmosphere (Torrell and Valles, 2001).

Efficiency with Artemisia Annua with neem oil: The experiment was carried out with another herbal oil that is known to be neem (*Azadirachta indica*), which represented in Figure 5. It was found that only one bite was there after 2h of the experiment. When extracted oil percentage was 20 and 30 there was 3 and 5 bites by

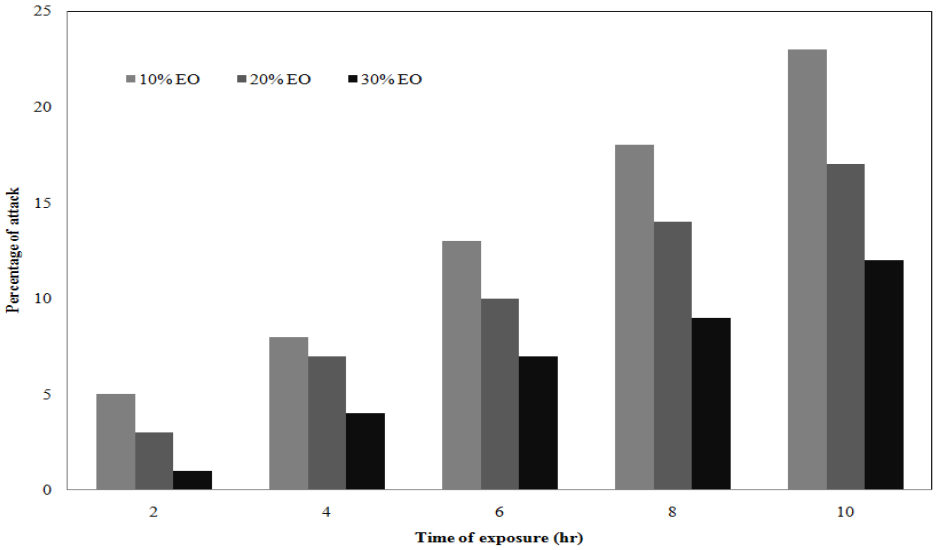

Figure 5. Effect of Artemisia Annua with neem oil

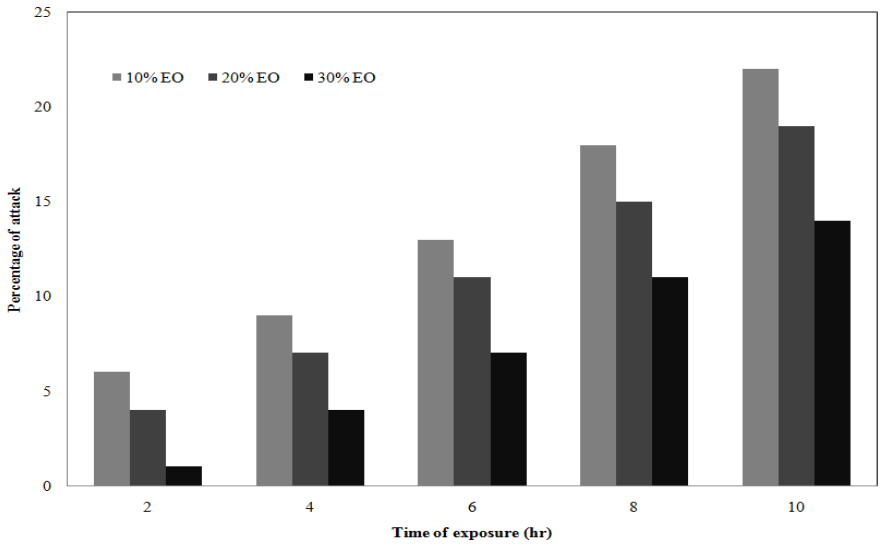

Figure 6. Effect of Artemisia Annua with rose oil

female *Aedes* (S.) *albopictus* mosquitoes. This was also increase with increase in time and decrease with percentage of extracted oil. This is mainly due to its main chemical composition is a blend of 3 to 4 related compounds along with over 20 lesser ones, which are equally as active. The general class of these compounds is triterpenes and within this category, the most effective are the limonoids, which are abundant in neem oil (Machroja et al., 2005).

Efficiency with Artemisia Annua with rose oil: The anti-repellent efficiency was carried out with rose oil at different percentages of extracted oil of AN, which represented in Figure 6. It was observed that there only single bite by

female mosquito upto 2h and it was increase 14 up to 10 hr of experimental time. When the percentage was 20 and 10 there was 4 and 6 bites by mosquitoes. Further increase in time upto 10 hr the bites increase up to 19 and 22 for 20% and 10% of extracted oil. The rose oil is come under the herbal and ascent. The constituting of rose oil containing 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives

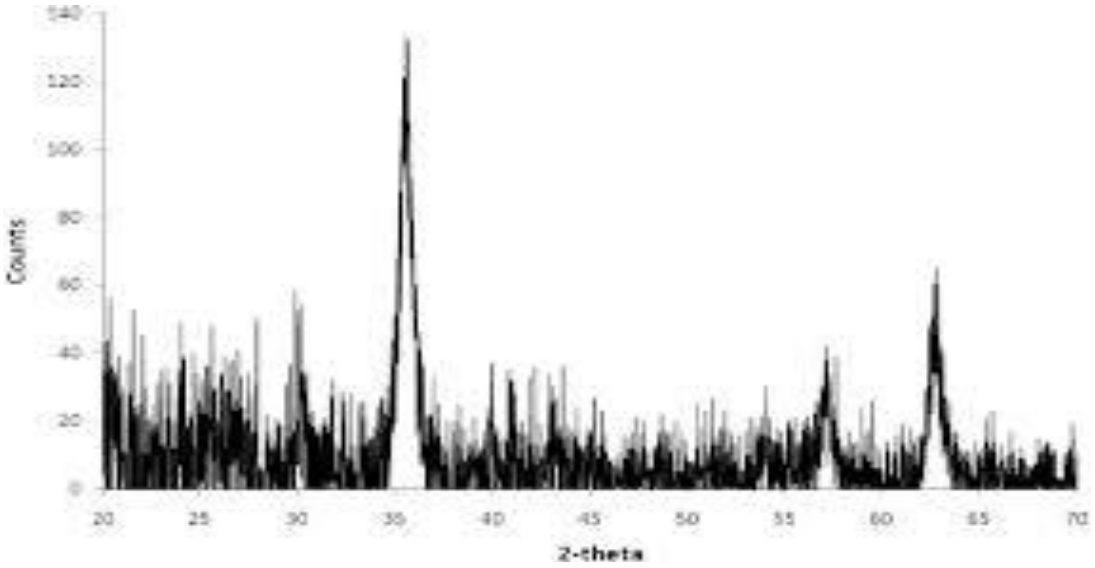

Figure 7. Composition oil in extracted oil from Artemesia annua L.

along with aliphatic aldehydes, alcohols, and esters (Zhang et al., 2009).

Gas chromatography study

Analysis of Essential Oil obtained after distillation was done using Gas Chromatography (GC)-Mass Spectrometer method, which shown Figure 7. The analysis shows the composition of the oil 1.2 % artemisinin content. Artemisinin, a sesquiterpene lactone containing a peroxide bridge, is isolated from the aerial parts of Artemesia annua L. plants and its derivatives are found effective against multidrugresistant P. falciparum strains (Jung et al., 1994). Since then malaria has been treated with quinoline based drugs. However, Plasmodium falciparum developed resistant globally against two of the most common ant malarial drugs: chloroquine and the combination sulphadoxine/pyrimethamine (Ferreira et al., 1995).In addition to potent anti-malarial activity, artemisinin posses anti-cancer (Efferth et al., 2001), herbicidal (Abdul-Jaleel et al., 2007), anti-hepatitis B (Romero et al., 2005), anti-HIV (Jung and Schinazi, 1994), anti-leishmanial (Aquil et al., 2009) and anti-schistosomiatic (Borrmann et al., 2001) activities.

CONCLUSION

It is concluding that medicinal plants with their biologically-active compounds may provide a viable alternative to synthetic anthelmintics, but sound scientific evaluation of such compounds is lacking. Artemisia annua is an effective antimalarial drug used currently in over 50 countries as the first line of treatment against chloroquine-resistant malaria. The extracted oil from Artemisia annua show good efficiency 30% concentration with eucalyptus oil for 2hours there not a single bite was observed. Extracted oil concentration of 30% with neem oil and rose oil shows only single bit in 2 hours. This proof that Artemisia annua extracted oil with eucalyptus oil has been effective for antimalarial repellents.

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