Essential oils extracted by steam and vacuum distillations from annatto seeds showed mosquito repellency and larvicidal activities [5, 6]. The steam distillation involved simple water extraction. The vacuum distillation involved hexane extraction and silica gel separation. The use of hexane solvent extraction, followed by hexane distillation, followed by silica chromatographic separation and hexane distillation again to obtain a "vacuum distillate" is essentially un-practicable, cost-prohibitive, and occupationally unsafe large use of inflammable solvent(s). Mosquito larvicidal activity with this vacuum distillate was higher than that of steam distillate; however, the repellent activity of vacuum and steam distillates was relatively similar. The essential oil in steam distillate had ishwarane (53%), beta-selinene (6%) and other minor components. The essential oil in vacuum distillate had geranylgeraniol (23%), ishwarane (17%) and other minor components. The other study showed hexane extract of annatto seeds provided better mosquito repellency compared with alcohol extraction [7].

Brief Summary

The present application focuses on insect repellency products which are spatial repellents (high volatility compounds) and/or contact repellents (intermediate volatility compounds). Spatial repellents have strong odor and are effective at a distance for protecting people from insects reaching proximity, especially mosquitoes. The most commonly employed spatial repellents in nature are volatile essential oils, C10 terpenoids. Contact repellents have weaker odor. Insects usually land on treated surface and are then repelled. Plant-based contact repellents may be C15 terpenoids.

In one aspect, the present disclosure refers to a composition comprising ishwarane and geranyl-α-terpinene. In another aspect, the present disclosure refers to a composition comprising geranylgeraniol and geranyl-α-terpinene. In yet another aspect, the present disclosure refers to a composition comprising two C15 compounds and three C20 compounds. In yet another aspect, the present disclosure refers to a method of repelling an arthropod comprising topical application of compound as described herein on the skin. In a further aspect, the present disclosure refers to a method of killing an arthropod comprising application of the composition as described herein. In a further aspect, the present disclosure refers to a method of repelling or killing an arthropod comprising topical application of the composition as described herein on the skin.

In one example of the disclosed composition, the composition comprises ishwarane,

15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol.

In one example of the disclosed composition, the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein concentration of ishwarane is from 5% to 25%, concentration of 15-hydroxy-a-muurolene is from 1% to 10%, concentration of geranyl-α-terpinene is from 10% to 25%, concentration of cembrene is from 1% to 15% and concentration of geranylgeraniol is from 5% to 35%.

In one example of the disclosed composition, the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein concentration of ishwarane is from 10% to 20%, concentration of 15-hydroxy-α-muurolene is from 3% to 17%, concentration of geranyl-α-terpinene is from 15% to 20%, concentration of cembrene is from 5% to 10% and concentration of geranylgeraniol is from 10% to 30%.

In one example of the disclosed composition, the composition comprises two C15 compounds and three C20 compounds.

In one example of the disclosed composition, the composition comprises two C15 compounds and three C20 compounds wherein the C15 compound is a sesquiterpenoid and the C20 compound is a diterpenoid.

In one example of the disclosed composition, the composition comprises two C15 compounds and three C20 compounds, wherein the sesquiterpenoid is selected from the group consisting of ishwarane, 15-hydroxy-α-muurolene; and the diterpenoid is selected form the group consisting of geranyl-α-terpinene, cembrene and geranylgeraniol.

In one example of the disclosed composition, the composition comprises two C15 compounds and three C20 compounds, wherein concentration of C15 compounds is from 1% to 40% and concentration of C20 compounds is from 10% to 70%.

In one example of the disclosed composition, the composition comprises two C15 compounds and three C20 compounds, wherein the concentration of C15 compounds is from 5% to 30% and concentration of C20 compounds is from 15% to 60%.

In one example of the disclosed composition, the composition comprises two C15 compounds and three C20 compounds, wherein the concentration of C15 compounds is from 10% to 20% and concentration of C20 compounds is from 20% to 50%.

In one example of the disclosed composition, the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein concentration of ishwarane is 20%, concentration of 15-hydroxy-α-muurolene is 5%, concentration of geranyl-α-terpinene is 22%, concentration of cembrene is 10% and

concentration of geranylgeraniol is 6%.

In one example of the disclosed composition, the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, further comprises at least one of peppermint oil, lemongrass oil, spearmint oil, cinnamon oil and oil of lemon eucalyptus

In one example of the disclosed composition, the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein concentration of ishwarane is 9%, concentration of 15-hydroxy-α-muurolene is 5%, concentration of geranyl-α-terpinene is 16%, concentration of cembrene is 3% and concentration of geranylgeraniol is 23%.

In one example of the disclosed composition, the composition comprises ishwarane and geranyl-α-terpinene.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application on the skin of the composition comprising ishwarane and geranyl-α-terpinene.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application on the skin of the composition comprising ishwarane and geranyl-α-terpinene, wherein the arthropod is selected from the group consisting of insect and arachnid.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application on the skin of the composition comprising ishwarane and geranyl-α-terpinene, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the insect is selected from the group consisting of mosquito and sandfly.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application on the skin of the composition comprising ishwarane and geranyl-α-terpinene, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the arachnid is a tick.

In one example of the disclosed composition, the composition comprises geranylgeraniol and geranyl-α-terpinene.

In one example of the disclosed method, the method of killing an arthropod comprises application of the composition comprising geranylgeraniol and geranyl- α-terpinene.

In one example of the disclosed method, the method of killing an arthropod comprises

application of the composition comprising geranylgeraniol and geranyl-α-terpinene, wherein the arthropod is selected from the group consisting of insect and arachnid.

In one example of the disclosed method, the method of killing an arthropod comprises application of the composition comprising geranylgeraniol and geranyl- α-terpinene, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the insect is selected from the group consisting of mosquito and sandfly.

In one example of the disclosed method, the method of killing an arthropod comprises application of the composition comprising geranylgeraniol and geranyl-a-terpinene, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the arachnid is a tick.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application of the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol on the skin.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application of the composition comprises ishwarane, 15-hydroxy-a-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol on the skin, wherein the arthropod is selected from the group consisting of insect and arachnid.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application of the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol on the skin, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the insect is selected from the group consisting of mosquito and sandfly.

In one example of the disclosed method, the method of repelling an arthropod comprises topical application of the composition comprises ishwarane, 15-hydroxy-α-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol on the skin, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the arachnid is a tick.

In one example of the disclosed method, the method of killing an arthropod comprises application of compound of the composition comprises ishwarane, 15-hydroxy-a-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol.

In one example of the disclosed method, the method of killing an arthropod comprises application of compound of the composition comprises ishwarane, 15-hydroxy-a-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein the arthropod is selected from the group consisting of insect and arachnid.

In one example of the disclosed method, the method of killing an arthropod comprises application of compound of the composition comprises ishwarane, 15-hydroxy-a-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the insect is selected from the group consisting of mosquito and sandfly.

In one example of the disclosed method, the method of killing an arthropod comprises application of compound of the composition comprises ishwarane, 15-hydroxy-a-muurolene, geranyl-α-terpinene, cembrene and geranylgeraniol, wherein the arthropod is selected from the group consisting of insect and arachnid, wherein the arachnid is a tick.

In one example of the disclosed method, the method of vacuum distillation to remove very volatiles (≤C10) with at least two distillation steps at ≤ 100 °C, and then distilled to obtain normal volatiles (C15 and C20) at> 100 °C.

In one example of the disclosed composition, blending of mono-, sesqui- and diterpenoids of annatto extracts provide an optimal effect.

In one example of the disclosed composition, annatto extracts have insect repellent potentials, where blending of mono-, sesqui- and diterpenoids of annatto extracts provide an optimal effect.

In one example of the disclosed composition, annatto extracts have larvicidal activity, where blending of mono-, sesqui- and diterpenoids of annatto extracts provide an optimal effect.

In one example of the disclosed composition, annatto extracts have various insect repellent potentials and contact killing, such as, larvicidal activity, where the ratio of monoterpenoids to sesquiterpenoids is 1:99 to 99:1.

In one example of the disclosed composition, annatto extracts have various insect repellent potentials and contact killing, such as, larvicidal activity, where the ratio of monoterpenoids to sesquiterpenoids is 25:75 to 75:25.

In one example of the disclosed composition, annatto extracts have various insect repellent potentials and contact killing, such as, larvicidal activity, where the ratio of monoterpenoids to diterpenoids is 1:99 to 99:1.

In one example of the disclosed composition, annatto extracts have various insect repellent potentials and contact killing, such as, larvicidal activity, where the ratio of monoterpenoids to diterpenoids is 25:75 to 75:25.

In one example of the disclosed composition, annatto extracts have various insect

repellent potentials and contact killing, such as, larvicidal activity, where the ratio of sesquiterpenoids to diterpenoids is 1:99 to 99:1.

In one example of the disclosed composition, annatto extracts have various insect repellent potentials and contact killing, such as, larvicidal activity, where the ratio of sesquiterpenoids to diterpenoids is 25:75 to 75:25.

In one example of the disclosed composition, annatto extracts have various insect repellent potentials and contact killing, such as, larvicidal activity, where the ratio of sesquiterpenoids to diterpenoids is 50:50.

In one example of the disclosed composition, annatto extracts have insect repellency and larvicidal potentials, where the ratio of ishwarane to geranyl-α-terpinene is 25:75 to 75:25.

In one example of the disclosed composition, annatto extracts have insect repellency and larvicidal potentials, where the ratio of ishwarane to geranyl-α-terpinene is 40:60 to 60:40.

In one example of the disclosed composition, annatto extracts have larvicidal activity, where the ratio of geranylgeraniol to total of sesquiterpenoids and diterpenoids is 1:100 to 99:100.

In one example of the disclosed composition, annatto extracts have larvicidal activity, where the ratio of geranylgeraniol to total of sesquiterpenoids and diterpenoids is 20:100 to 90:100.

In one example of the disclosed composition, annatto extracts have larvicidal activity, where the ratio of geranylgeraniol to total of sesquiterpenoids and diterpenoids is 40:100 to 60:100.

In one example of the disclosed composition, the combination of annatto extracts and an essential oil selected from the group of peppermint oil, lemongrass oil, spearmint oil, cinnamon oil, oil of lemon eucalyptus, containing different ratios of composite monoterpenoids, sesquiterpenoids and diterpenoids have synergistic effects.

In one example of the disclosed composition, annatto extracts with around 0.1 - 1.0% tocotrienols have antioxidant shelf life extension for insect repellent and larvicides.

In one example of the disclosed composition, annatto extracts with insect repellency and larvicidal potentials are in aerosol sprays, lotions, creams, liquids, wristbands, wipes, and candles.

In one example of the disclosed composition, annatto extracts with insect repellency and larvicidal potentials comprise a combination of geranyl-α-terpinene and

geranylgeraniol.

In one example of the disclosed composition, preparations with enhanced insect repellency and larvicidal activity contain combinations of annatto extracts and oil of lemon eucalyptus.

In one example of the disclosed composition, preparations with enhanced insect repellency and larvicidal activity contain combinations of annatto extracts and oil of lemon eucalyptus at a ratio of 50:30.

In one example of the disclosed composition, preparations with enhanced insect repellency and larvicidal activity contain combinations of annatto extracts and DEET.

In one example of the disclosed composition, preparations with enhanced insect repellency and larvicidal activity contain combinations of annatto extracts, oil of lemon eucalyptus, and DEET.

In one example of the disclosed composition, preparations with enhanced insect repellency and larvicidal activity contain combinations of annatto extracts and triglyceride oils.

In one example of the disclosed composition, a natural emulsifier quillaja is added to reduce surface tension to provide a wide but thin surface layer on water bodies for larvicidal property.

In one example of the disclosed composition, the composition comprises a combination of medium chain length fatty acids from coconut oil and annatto extracts have synergistic effects on insect repellency and larvicides.

In one example of the disclosed method, the method of vacuum distillation obtains a higher portion of diterpenoids (C20) and a lower portion of sesquiterpenoids (C15).

In one example of the disclosed method, the method of vacuum distillation removes very volatiles (≤C10) with at least two distillation steps at :≤100 °C, and then distilled to obtain normal volatiles (Cl5 and C20) at> 100 °C.

In one example of the disclosed method, the method of vacuum distillation is solvent free process.

In one example of the disclosed method, the method of vacuum distillation uses a pressure around 0.02 torr.

In one example of the disclosed method, the method of vacuum distillation has a 2- 3 passes.

In one example of the disclosed method, the method of vacuum distillation obtains AOE 1, AOE 2 and AOE 3 from the first and/or the second pass.

In one example of the disclosed method, the method of vacuum distillation obtains tocotrienols from the third pass.

In one example of the disclosed method, the method of vacuum distillation the distillation condition for pass 1 and/or 2 is 120° C - 130° C and 0.03 - 0.08 torr and the distillation condition for pass 3 is 198°C - 210°C and 0.01-0.09 torr.

In one example of the disclosed method, the method of vacuum distillation to obtain annatto oil extracts uses up to total 5 passes.

In one example of the disclosed method, the method of vacuum distillation The condition in pass 1 may be around 120°C - 160°C and vacuum may be around 0.03 - 2 torr.

In one example of the disclosed method, the method of vacuum distillation, the condition in pass 2 is around 160°C - 170°C and vacuum may be around 0.03 - 0.6 torr.

In one example of the disclosed method, the method of vacuum distillation obtains AOE 1 from pass 1 and AOE 2 and 3 from pass 2.

In one example of the disclosed method, the method of vacuum distillation to obtain high concentrations of a specific compound, such as, geranylgeraniol (90% in AOE 3) pass 2 is repeated several times.

In one example of the disclosed method, the method of vacuum distillation obtains differences between AOE 1, AOE 2 and AOE 3 by changing the process.

In one example of the disclosed method, the method of vacuum distillation obtains compounds in AOE 3 which are 90% geranylgeraniol (diterpenoid) with higher molecular weight from process with higher temperature and vacuum.

In one example of the disclosed method, the method of vacuum distillation obtains compounds in AOE 2 of intermediate molecular weight from process with intermediate temperature and vacuum.

In one example of the disclosed method, the method of vacuum distillation obtains the compounds in AOE 1 with lower molecular weight from process with lower temperature and vacuum.

In one example of the disclosed method, the method of vacuum distillation allows repeatable production of AOE 1, AOE 2 and AOE 3.

In one example of the disclosed method, the method of vacuum distillation in pass 3 -

5 the major component of annatto extract is tocotrienols.

In one example of the disclosed method, the method of vacuum distillation in pass 1 - 2, the major components are terpenoids with lower molecular weight, since the process is milder.

In one example of the disclosed method, the method of vacuum distillation focuses on the terpenoids with lower molecular weight compared with tocotrienols by using milder process.

In one example of the disclosed composition, tocotrienols are used as antioxidants to extend shelf life of insect repellent and larvicides.

In one example of the disclosed method, the method of vacuum distillation obtains annatto oil extracts with monoterpenoids by using milder a process, such as, lower temperature and vacuum.

In one example of the disclosed method, the method of vacuum distillation obtains concentrations from 3% to 30% of monoterpenoids from annatto oil extract with a milder process.

In one example of the disclosed method, the method of vacuum distillation produces the composition with major monoterpenoids of α-pinene, β-pinene, camphene, limonene, myrecene, cis-ocimene, chrysanthenone, and eucarvone.

In one example of the disclosed composition, the monoterpenoids, specifically a-pinene, B-pinene, camphene, limonene and myrecene, have insect repellent potential and larvicidal activity.

In one example of the disclosed composition, the combination of monoterpenoids, and sesquiterpenoid and diterpenoid in AOE 1 has strong insect repellent potential.

In one example of the disclosed composition, the combination of these monoterpenoids, and sesquiterpenoid and diterpenoid of AOE 2 have strong larvicidal activity.

In one example of the disclosed composition, the ratio of ishwarane to geranyl-a-terpinene in AOE 1 has strong insect repellency.

In one example of the disclosed composition, the ratio of ishwarane to geranyl-a-terpinene in AOE 2 has strong larvicide activity.

In one example of the disclosed composition, the annatto oil extract has

diterpenoids in the range from 40% to 75% and has sesquiterpenoids in the range from 13% to 45%.

In one example of the disclosed composition, AOE 3 has 90% geranylgeraniol. In one example of the disclosed composition, AOE 3 has 91% of diterpenoids.

In one example of the disclosed composition, the concentration of geranylgeraniol to sesquiterpenoids and diterpenoids is 99%.

In one example of the disclosed composition, AOE 2 has 23% geranylgeraniol.

In one example of the disclosed composition, AOE 2 has 16% of sesquiterpenoids and 74% of diterpenoids.

In one example of the disclosed composition, the concentration of geranylgeraniol to sesquiterpenoids and diterpenoids is 26%.

In one example of the disclosed composition, AOE 1 has 6% geranylgeraniol.

In one example of the disclosed composition, AOE 1 has 38% of sesquiterpenoids and 52% diterpenoids.

In one example of the disclosed composition, the concentration of geranylgeraniol to sesquiterpenoids and diterpenoids is 7%.

Having described example s of the disclosed compositions and methods, it will now become apparent to those of ordinary skill in the art that other example s incorporating these concepts may be used. Accordingly, it is submitted that the disclosed compositions and method should not be limited to the described example s but rather should be limited only by the spirit and scope of the appended claims.

Brief Description of the Several Views of the Drawing

Figure 1 shows a Y-tube olfactometer. Figure 2 shows a sample with hand.

Figure 3 shows a control with hand.

Figure 4 shows the Y-tube results: percent attraction per treatment. AOE is annatto extract. DEET is *N,* N-diethyl-3-methylbenzamide. OLE is oil of lemon eucalyptus. The control was a cotton ball treated with mineral oil.

Figure 5 illustrates the experimental design and cartoon representation of larvicidal studies.

Figure 6 illustrates the larvicidal activity of annatto extract 1 (AOE 1) after 1 hour post exposure. Y-axis represents the percent mortality after 1 hour of exposure to AOE 1. X-axis represents the amount of the AOE 1 added to 200 mL of distilled water.

Figure 7 illustrates the larvicidal activity of annatto extract 1 (AOE 1) after 24 hours post exposure. Y-axis represents the percent mortality after 24 hours of exposure to AOE 1.

X-axis represents the amount of the AOE 1 added to 200 mL of distilled water.

Figure 8 illustrates the larvicidal activity of annatto extract 1 (AOE 1) after 24 hours post exposure. Logistical regression of killed by log (Dose (µl)) at 24 hours.