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Evaluation of Chemical Composition and the Comparative Wound Healing Effect of Natural Honey and Olive Oil in Rabbits

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Abstract. The phytochemical and elemental constituents of honey and olive oil and their relationships with wound healing were studied in New Zealand rabbits. Phytochemical analysis showed the presence of Carbohydrates, monosaccharide, reducing sugar, terpenoid, saponin, saponin glycoside, flavonoids, and alkaloid in honey, while olive oil had only cardiac glycoside and terpenoids. Elemental analysis showed the presence of higher concentration of potassium (288.3mg/l), and low concentration of manganese (0.04mg/l), iron (1.55mg/l), copper (0.66mg/l) and nickel (0.001mg/l) in honey than olive oil. However, olive oil has higher concentration of calcium (225.3mg/l) and zinc (5.22mg/l) than in honey. The wound healing effects of honey and olive oil were determined by inflicting a 2 mm incisional wound on the thigh muscle of 18 New Zealand rabbits. The rabbits were grouped into six groups (A-F) which were topically administered of honey (A), olive oil (B), honey and olive oil mixture (C), iodine and methylated spirit (D) and penicillin (E).Group F was undressed and untreated and served as negative control. Healing and scar tissue formation was monitored over a period of 10 days by measuring the wound closure daily. It was observed that group B and D had complete resolution with minimal scar tissues by day six. Other groups had a longer resolution time of up to 9 days post incision. The study therefore, showed olive oil alone and a combination of iodine and methylated spirit both aided greatly in dehiscence of uncontaminated surgical wounds in New Zealand rabbits.

Keywords: Incision wound, uncontaminated, scar, rabbits, olive oil, honey.

Introduction

Traditional management of wounds involves the use of herbs powder as a whole or their byproducts as plant extracts preparations such as olive oil extracted from the seed of olive tree. Olive oil has been considered sacred and was used to anoint kings and athletes in ancient Greece (Balfour and John, 1881). These byproducts were used too in wound dressing (Bergman *et al.,* 1983), and treatment of diseases in humans such as diaper, dermatitis, psoriasis, eczema and other fungal skin infections (El-Okdi *et al.,* 2008).These are topically applied either singly or in combination with other agents such as honey (Standifer,1993) a natural byproduct produced by honey-bee. However, honey is more frequently used in management of sore wounds and burns. Historically, it was used to treat infected wounds for over 2000 years before bacteria was discovered as a clear cause of infection (Grunther, 1934)

Wounds can be defined as a breach in the continuity of the skin and some underlying tissues of the body caused by a sharp object which may injure or damage the dermis of the skin (Thornton, 2004). Wounds may be classified based on the nature of the wound as open (incised, laceration, penetration and abrasion) or closed (contusions, hematomas and crush injuries) wound (Abuhafeil *et al.,* 2006).However, wound healing in all these types of wounds undergo same process, and is the restoration of normal anatomical continuity of a disrupted area of tissue.

Wound healing is a complicated and important process, through depending on the initiation of arrest of hemorrhage (homeostasis) which is a reaction between thromboplastin from damaged tissues, the platelets and the circulating prothrombin and calcium to produce fibrin. These threads of fibrin form a sea of folds which hardens into a scab (matrix) that bridges the gap and under which tissue repair can commence (Wahl and Wahl 1992).

Many synthetic agents have been used in aiding wound healing process and equally, to reduce wound infection by microbes. These synthetic agents are developed for parenteral and topical applications. However, associated with these synthetic agents are drawbacks such as increase in wound healing time and scab formation

after wound healing, development of resistance to some antibiotics by some microbes (Browder *et al.,* 1998) associated with abundant therapeutically inactive drugs in the markets, necessitate re-evaluation of ancient wound healing remedies such as honey and olive oil (Molan, 1992).

Honey is a supersaturated viscous fluid (2-10 kilo Pa.s) with molecular formula $C_{12}H_{22}O_{11}$ (White, 1984). The elements carbon, hydrogen and oxygen are covalently bonded (Barry, 1999). Honey basically, contain fructose and glucose with 1 % sucrose and 17 % water; it also has less than 10 % contents of other sugars (Tomasik, 2004). Honey has a melting point of $40^0 – 50^{0C}$ and rapidly crystallizes at temperatures between 13^{0C} and 17^{0C}. Pure honey is acidic and has a pH of 3.9 and range of (3.4 -6.1), it has a density of 1.36 kg which is 36 % denser than water, and a glycemic index (GI) between 31- 78 (Krell, 2011). Pure honey is usually distinguished by its fragrance, taste and consistency; freshly collected honey should flow from knife edge without breaking drops (White and Landis, 1980), (Figure 1).

Olive oil is naturally occurring oil extracted from the olive tree (Tous and Ferguson, 1996, Babio *et al.,* 2009). It is a saturated fatty acid with basic molecular formula $CH_3(CH_2)$ nCOOH, (where 'n' is an even number of carbon atoms contained in the chain and normally range between 16 to 28) and a carboxylic group COOH (Mailer, 2007). It consists mainly of glycerol, free fatty acid and triglycerides. Olive oil is reported to contain saturated, monounsaturated and polyunsaturated fatty acids (Liu, 1999), 58-83 % Oleic acid, 5.5-21 % linoleic acid (Mailer, 2006). It is also containing 7.5-20 % palmitic acid, 0.5- 5 % stearic acid and 0.1-5 % linolenic acid (Betram *et al.,* 2004). Olive oil has a melting point of $– 6^{0C}$ and a boiling point of 300^{0C}, its specific gravity is 75-95 with a refractive index of 1.4677- 1.4705 at 15.5^{0C}. It is a viscous liquid with saponification value of 184-196 and freezes at $37\text{-}39^0$C .It has an acid value of 6.6 (Bianco *et al.,* 2007), (Plate 1 and plate 2).

Honey have been reported to have inhibitory effect on 60 species of both aerobic and an aerobic, Gram positive and Gram negative bacteria (Ali, 1991) through the activities of hydrogen peroxide produced by the honey (Bunting, 2001), equally, it is reported to have anti-fungal activity (Molan, 1992), while oils from olive tree fruits

are also demonstrated to have activity on some bacteria and fungi and enhancement of wound healing (Bergman *et al.,* 1983), Most of the wounds treated with these agents (honey and olive oil) are burns (Postmes *et al.,* 1997; Subrahmanyam, 2004) or sores (Molan, 1992; Kerstein, 1997). With dearth of information on incised wound treatment with these agents therefore, this work is designed to evaluate wound healing effect of both honey and olive oil, and the phytochemical constituents responsible for these effects.

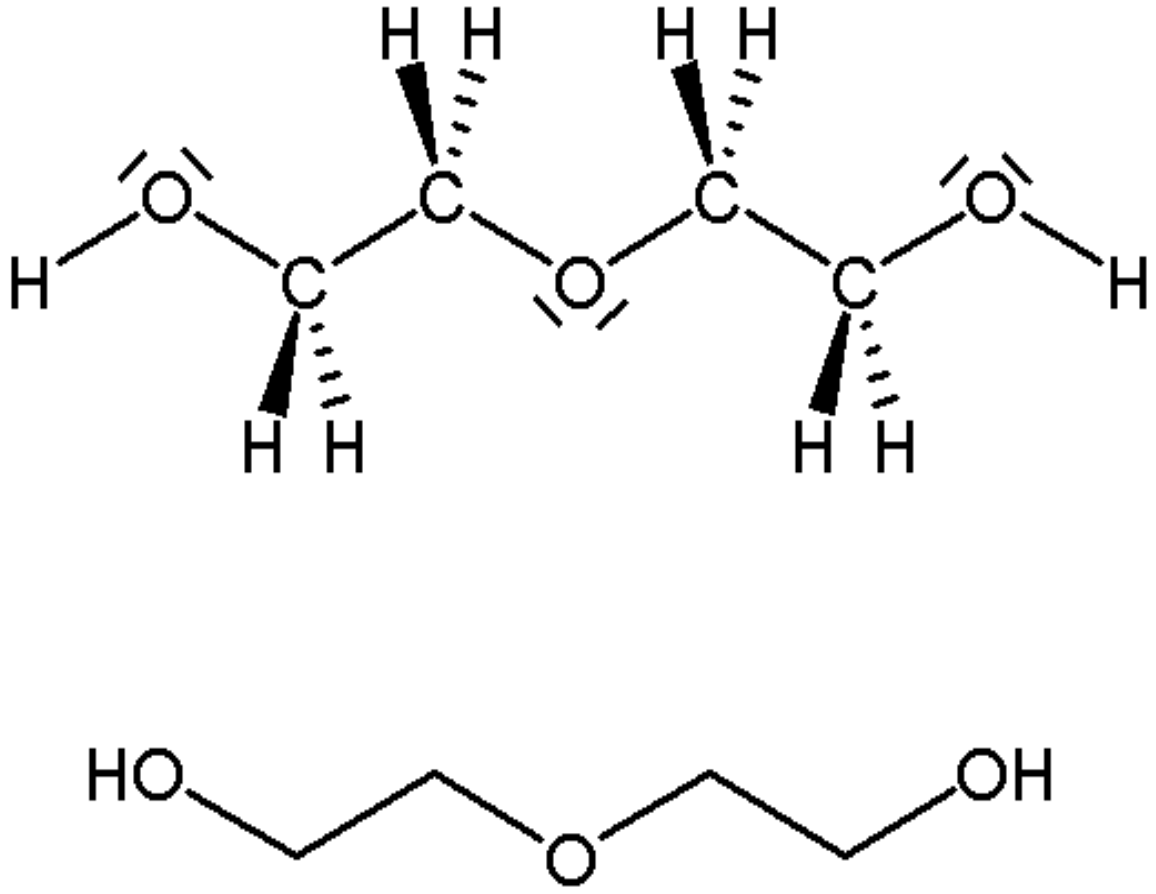

Figure 1: Molecular structure of honey (Raymond, 2010).

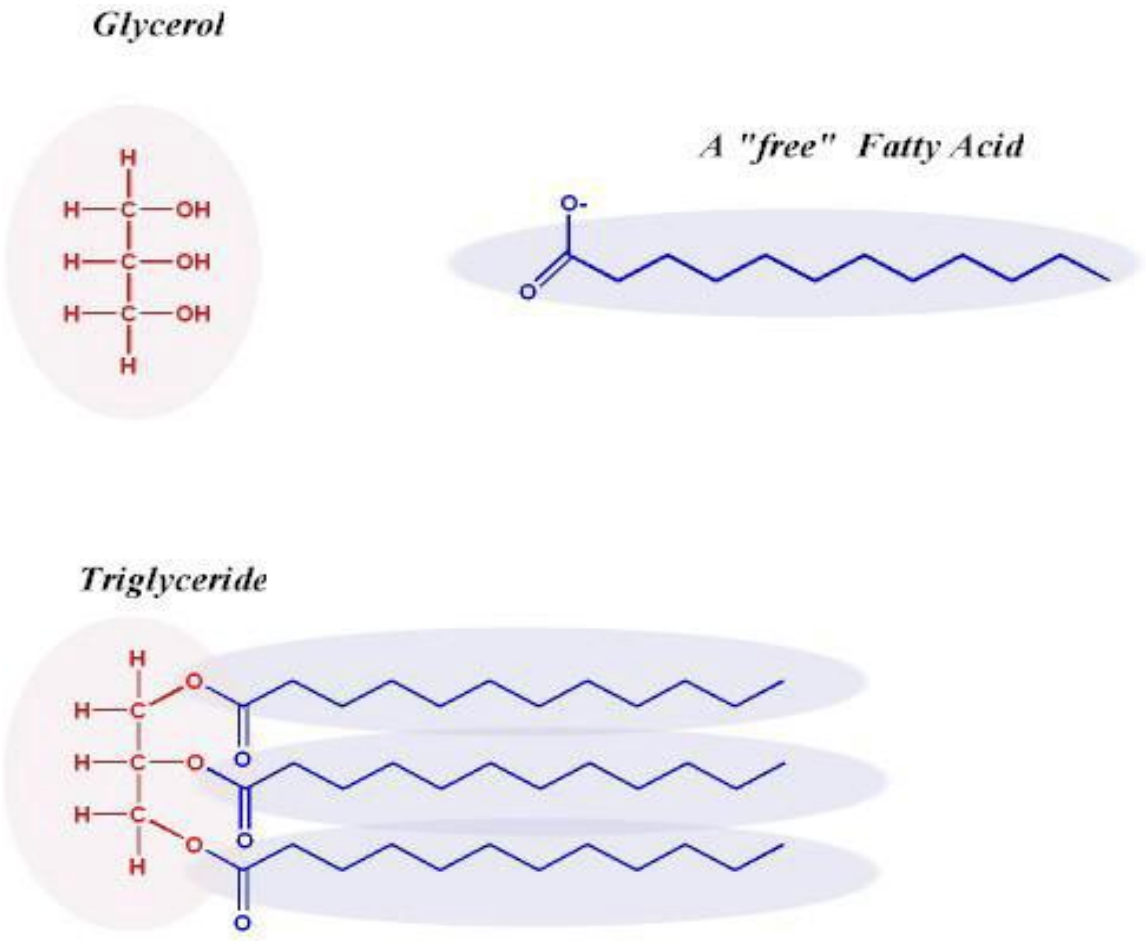

Plate 1: Schematic structures of Glycerol, fatty acids and triglyceride in olive oil (Mailer, 2006)

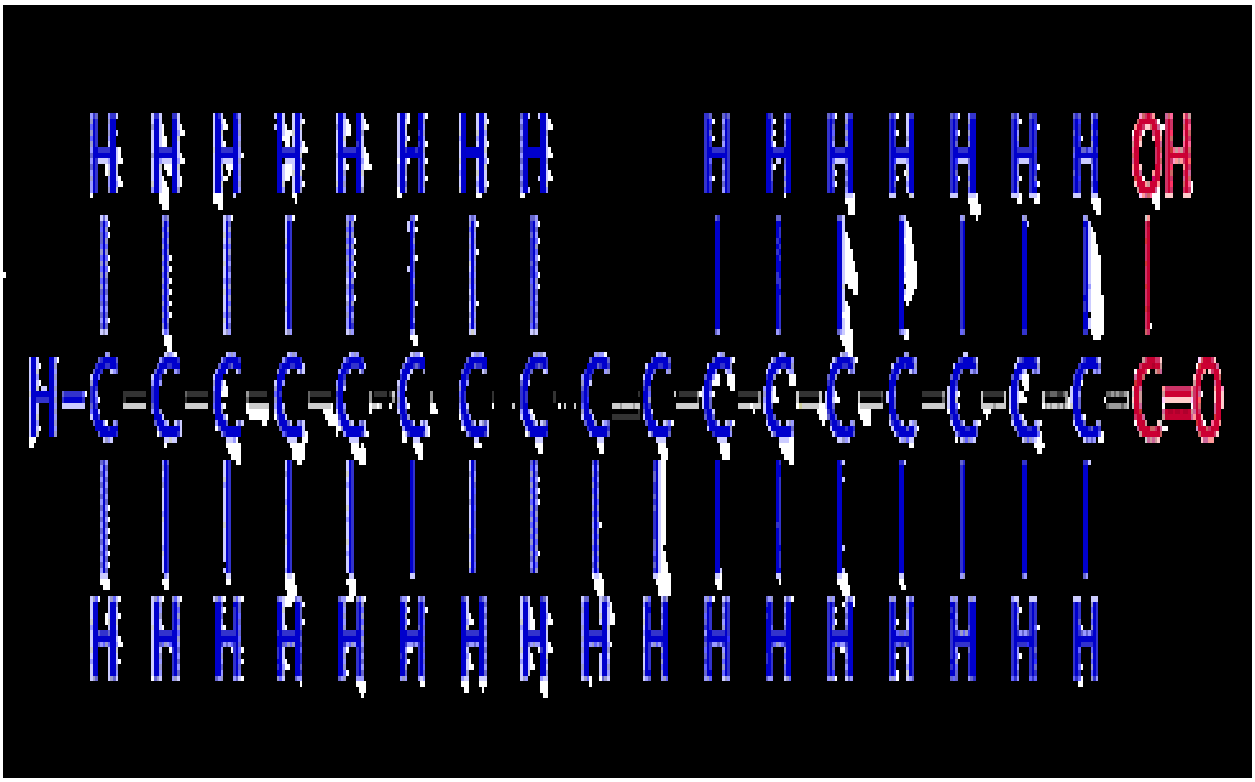

Plate 2: Molecular structure of olive oil (Bianco *et al.,* 2007)

Materials and Methods

Animals

Rabbits

A total of eighteen New-Zealand breed of rabbits aging between 3 and 4 months and of both sexes, with an average weight of 3.0kg, were purchased from Maiduguri Monday market. These rabbits were divided into six groups (A-F) of three rabbits/group, they were kept for two weeks to acclimatize, in separate metal cubicle and fed with green vegetables and corn chaft, while water was provided *ad libitum*. The cubicles were regularly disinfected with household bleach and kept clean; the rabbits were closely monitored for any clinical signs of disease before commencement of the experiment.

Honey

Fresh honey was obtained from local honey tapper at Bwari local government area in Abuja, the Federal Capital Territory, Nigeria. Purity of honey was determined by using the cotton wick test (Harihar, 2007)

Olive oil

Extra virgin olive oil produced by Yudun Gida (Emir, Trihalat-ihticat ve Tlc, Ltd. Antakya, Turkey) was purchased from (Today super stores) at supermarket in Maiduguri, Borno State, Nigeria.

Lidocaine hydrochloride solution, tincture of iodine, methylated spirit and penicillin ointment

Lidocaine hydrochloride (Sensinil^{R}, 2 %) solution for local infiltration produced by Claris lifesciences limited Chachawadi Vasana Ahmadabad, India. Marketed by Java international limited, Isolo Industrial Estate, Lagos Nigeria.)Tincture of iodine (Jovic Enterprise, Agbo, in Abia State, Nigeria), methylated spirit made of alcohol 95% v/v and 5% Naphthalene, (Gauze Pharmaceuticals and laboratories Ltd, Awka, Nigeria) and penicillin ointment 0.1 mg/g penicillin potassium B.P produced by MIM Pharma. Ind. Ltd. Owode-Idiroko Rd. Ola, Ogun State, was purchased from a local chemist shop (Array Pharmacy, Lagos Street) in Maiduguri. Borno State, Nigeria.

Methods

Treatment

Each rabbit was restrained by handling. The incision sites were shaved with a razor blade and local infiltration around the site of incision was made with 0.03 ml Lidocaine hydrochloride solution at a dosage 1 mg / 5 kg and a concentration of 20 mg/ml. A 2 mm incision was made on the dorsal aspect of the left thigh with a clean aseptic scalpel blade on rabbits in all the groups. The incised wound was then cleaned using cotton wool draped in methylated spirit in groups A, B, C, E and F, while rabbits in group D were treated with iodine to irrigate and stop bleeding from the incised wound. Subsequent treatment in this group (D) continued with methylated spirit dressing. Treatment with the test agents commenced as soon as

bleeding stopped. At a dosage of 10 mg/kg of the treatment agents, a concentration of 200 mg/ml of honey and olive oil were prepared. A dose of 0.2 ml of the prepared honey was topically administered to rabbits in Group A, and rabbits in group B were treated with 0.2 ml of olive oil, while rabbits in group C were treated with a combination of honey and olive oil (0.2 ml) mixed in equal proportion (1:1), The rabbits in group D were treated by dressing the wound with only iodine and methylated spirit on the first day, and subsequently dressed with methylated spirit for the period of the experiment, group E were treated by applying synthetic penicillin ointment (0.1 mg/g, w/w) and considered as positive control, while rabbits in group F were left undressed and untreated as negative control group.

Phytochemical studies

These tests were conducted with 5mls of both honey and olive oil using methods described by Harbone (1976). Phytochemicals such as Carbohydrates, monosaccharide, reducing sugars, combined reducing sugars, soluble starch, ketones, pentoses, tannins, phlobatannins, free anthraquinones, glycosides, cardiac glycosides, saponins, saponins glycosides, flavonoids and alkaloids were analysed.

Elemental analyses

The elemental analysis in both honey and olive oil was analyzed using the Winlad 32 software for the atomic absorption spectrophotometer (AAS). The analysis was done to assess for elements such as calcium, manganese, iron, copper, cobalt, selenium, zinc, lead and nickel, while potassium was determined by flame emission spectrophotometry.

Determination of incised wound regression

Wound healing (regression) was assessed by using a vernier caliper (Mitutoyo Mfg. Co. Ltd. Tokyo, Japan) to measure wound size with time in mm/day. The experiment lasted for a period of ten days.

Statistical analysis

Data were presented as mean ± SD and analyzed by one way analysis of variance (ANOVA) using Graphpad InStat version 3.00 for 95 Software (2003). $P<0.05$ values were considered significant and the results presented In Table III, and as linear graph in figure 1.

Results

Phytochemical analysis of honey reveals the presence of Carbohydrates, monosaccharide, cardiac glycosides, saponins, flavonoids, alkaloids and terpenoids, while in olive oil, cardiac glycoside and terpenoids were the phytochemicals detected (Table I).

Elemental analysis for honey reveals the presence of Calcium (189.0mg/ml), Manganese (0.04mg/ml), Potassium (388.3mg/ml), Iron (1.550mg/ml), Copper (0.66mg/ml), Zinc (2.48mg/ml) and Nickel (0.001mg/ml), however, Cobalt, Selenium and Lead were not detected (Table II).

Elemental analysis for olive oil showed that Calcium was (225.3mg/ml), Manganese (0.02mg/ml), Potassium (271.5mg/ml), iron (0.93mg/ml), Copper(0.41mg/ml) and Zinc (5.22mg/ml), but element such as Cobalt, Selenium, Lead and Nickel were not detected (Table II).

The values of calcium and potassium, recorded in both honey and olive oil were significantly higher than the recommended standards (3.6-80 mg/ml and 0.01-0.1 mg/l) World Health Organization (1993), while iron was slightly higher in honey approximately (1.6 mg/ml),and olive oil (0.93 mg/ml) when compared to 0.05-0.6 mg/ml rate by WHO standards, manganese in both honey and olive oil were lower (0.04 mg/ml and 0.02 mg/ml) than the recommended WHO standards (0.1-20 mg/ml), Copper in the agents studied recorded slightly higher values (0.7mg/ml and 0.4 mg/ml) than WHO standards approximately (0.1-0.3 mg/ml), Cobalt, Selenium and Lead were not detected therefore fell short of WHO standards (0.1-0.2 mg/ml, 0.1-2.0 mg/ml and 0.005-0.03 mg/ml) while Zinc in both honey and olive oil were found to be within normal WHO standards, (2.48 mg/ml and 5.22 mg/ml). However, Nickel at (2-5 mg/ml) was negligible in honey and was not detected in olive oil. (Table II).

Incised wounds treated with honey showed wound regression by the 2nd day post treatment and healing was completed by the 9th day (Table III, figure 1). Groups treated with olive oil showed remarkable wound healing effect as wound regression was observed from 3rd day post treatment while wound healing was completed by 6th day (Table III, figure 1). Delayed wound healing was noticed in the group treated

with mixtures of honey and olive oil, healing started on the 3rd day and lasted for 7 days, total cure was achieved by the 8th day. However, the group (E) treated with conventional synthetic drug penicillin, was totally healed on the 10th day. Wound healing in iodine and methylated spirit treated group commenced on the 2nd day post treatment and lasted for 5 days with total cure on the 6th day. In the untreated group, wound healing started on the 2th day post incision and was 0.2 mm by the 10th day of the experiment. Complete wound healing was achieved beyond the 10 day experimental period.

The groups that were treated with olive oil showed little scar formation when compared to other treatment groups in this study.

7. Table I: Quantitative phytochemical contents found in honey and olive oil.

Carbohydrates (Molisch's test)	+	_
Monosaccharide (Barfoed test)	+	_
Reducing sugar (Fehling test)	+	_
Combined reducing sugar	+	_
Soluble starch	_	_
Ketones (Saliwanorff's)	_	_
Pentoses	_	_
Tannins ($FeCl_2$, Pb acetate & HCl)	_	_
Phlobatannins	_	_
Glycosides	_	_
Anthraquinones (Borntrager's)	_	_
Cardiac glycosides (Salkowski's & L-Bucharnard's)	_	+
Saponin glycoside (Frothing & Fehling)	+	_
Terpenoids	+	+
Saponins (Shinoda;s)	+	_
Flavonoids	+	_
Alkaloids (Dragendorff's & Meyer's)	+	_

Key: + = Present, - = Absent

Table II: Result of elemental constituents of honey and olive oil.

Parameter	WHO standards (mg/l)	Honey (mg/l).	Olive oil (mg/l).
Calcium	3.6-80	189.0	225.3
Manganese	0.1-20	0.04	0.02
Potassium	0.01-0.1	288.3	271.5
Iron	0.05-0.6	1.550	0 .93
Copper	0. 1-0.3	0.66	0.41
Cobalt	0.1-0.2	0.00	0.00
Selenium	0.01-2.0	0.00	0.00
Zinc	0.15-20	2.48	5.22
Lead	0.005-0.03	0.00	0.00
Nickel	2.0-5.0	0.001	0.00

1ppm = 1mg/kg (WHO, 1993)

Table III: Incised wound size measurement in rabbits 1-10 days after treatment with honey, olive oil and their combination

Treatment agent	Groups	Days of treatment 1	2	3	4	5	6	7	8	9	10
Honey	A	0.8±0.1	0.7± 0.1	0.5± 0.2	0.4± 0.3	0.3±0.3^{x}	0.2± 0.2^{a}	0.1± 0.2^{b}	0.1± 0.2^{b}	0^{c}	0
Olive oil	B	0.4± 0.1	0.4± 0.1	0.3± 0.2	0.2± 0.2	0.1± 0.2	0^{a}	0x^{b}	0^{b}	0^{b}	0
Olive oil + honey	C	0.5± 0.1	0.5± 00	0.4± 0.1	0.3± 00	0.3± 0.1^{a}	0.1± 0.1^{b}	0.1± 0.1^{b}	0^{c}	0^{c}	0
Iodine + M. spirit	D	0.6± 0.2	0.5±0.2	0.3± 0.1	0.2±0.2	0.1±00	0 ^{c}	0 ^{c}	0	0	0
Penicillin	E	0.6± 0.2	0.5± 0.3	0.4± 0.2	0.3±0.1	0.3±0.1	0.3± 0.1	0.3± 0.1	0.3± 0.1	0.3±0.1	0^{c}
Untreated	F	0.6± 00	0.5± 00	0.5± 00	0.5± 00	0.4± 00	0.3±00	0.2±00	0.3±00	0.2±00	0.2

All measurement are in millimeters (mm).

P<0.05 = considered significant compared to control

Key : ^{a} = Significant, ^{b} = Very significant, ^{c} = Highly significant, 0 = Total healing

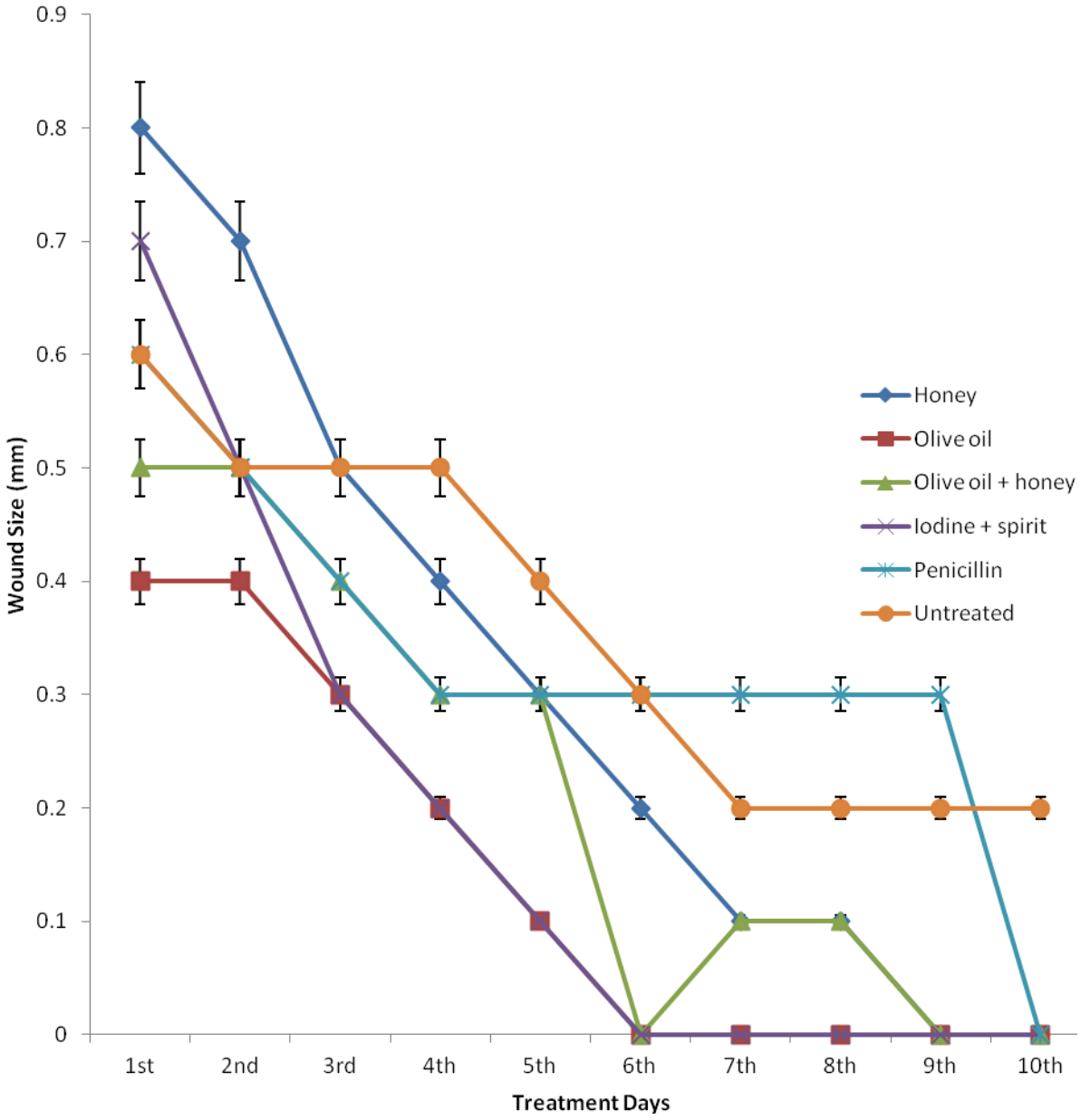

Figure 1 : Incised wound size measurement in rabbits 1-10 days after treatment with honey, olive oil and their combination.

Discussion

In this study, the wound healing effect of honey and olive oil can be related to high concentrations of elements such as calcium, potassium, iron, copper and zinc (in honey and olive oil) and phytochemical components such as carbohydrates, monosaccharide, reducing sugars, combined reducing sugars, terpenoids, saponins, flavonoids and alkaloids in honey. Cardiac glycoside and terpenoids in olive oil observed. These elements were reported to positive inotropic effect with increase in blood flow in the system and alteration of osmotic gradient around microbes respectively. This agrees with the finding of Bran and Turner (1975) that high concentration of some elements and contents in honey and olive oil have fastening effect on wound healing.

The study revealed that more phytochemicals are found in honey compared to olive oil, this could be due to the fact that honey bees processed nectars from various flowers found in different soil type (Krell, 2011).

Wound (regression) healing in this study is shown to start spontaneously on the 2nd day post incision except the groups treated with olive oil alone and those treated with mixtures of olive oil and honey that showed wound regression by the 3rd day. This supports the findings of Schoner and Scheiner-Bobis *(*2007) that reported downward alterations in salt metabolism and cell growth, due to presence of elements and phytochemicals such as potassium and flavonoids. The wound healing observed in the honey treated group in this study could be attributed to presence of carbohydrate in the honey. It was reported that carbohydrates enhance wound healing and repair by promoting early mobilization of macrophage to wound area (Wolk *et al.,* 1985; Standifer, 1993).This periwound migration of the macrophages increase fibroblast proliferation, fibrogenesis, collagen synthesis, epithelization leading to an increase in the tensile strength of the wound surface (Wolk *and danon,* 1985). This action is presumed to be the effect of glucan induced interleukin-1 (IL-1) and tumor necrotic factor-α (TNF-α) on the wound fibroblast (Browder *et al.,* 1988, Tonks *et al.,* 1979). Similarly, Kaplan (1984) opined that carbohydrate extracts from yeast accelerate open wound healing by stimulating oxygen consumption, increase angiogenesis and collagen synthesis in the wound. Monosaccharides were reported

to also have antibacterial property (Vaughan, 2001; Root and Root, 2011). These antibacterial property of monosaccharide as found in this study can prevent the wound infection and facilitate fast wound healing. Alkaloids were equally reported to have anti-bacterial activity (Molan, 1992; Idowu *et al.,* 2003). Terpenoids of plant origin are equally reported to have antibacterial (Zwenger and Basu, 2008) and anti-inflammatory activity (Loggia, 2000). These anti-inflammatory activities of terpenoids were attributed to a tryptophan derivative- Kinuremic acid, which has inhibitory effect on ionotropic receptor (Laddawan *et al.,* 2009).This may play a role in enhancing wound healing as observed in this study. Similarly, Ikari (2004) and Sundaran *et al.,* (1999) reported that terpenoids have regulatory effect on intracellular distribution of Na^+ dependent glucose transporter, hence playing a role in recovery from cellular injuries, (de las Heras and Hortetano, 2009). Honey equally is reported to exert osmotic effect, (Chirife *et al.,* 1993) and increase phagocytic and lymphocytic activity (Abuhafeil 1999). Additionally, honey also have an enzyme that produces hydrogen peroxide (White *et al.,* 1963, Bunting, 2001) and these can reduce wound contamination and enhance fast healing effects (Postmes *et al.,* 1997).

The wound healing effect of olive oil in this study and the reduced scar formation observed could be attributed to its cardiac glycoside constituents. A report by Everist (1974) has attributed the effect of bufadienodide, a cardiac glycoside to have anti- scar effect This coupled with high Zinc and Calcium elemental concentration, could be responsible for the enhanced wound healing effect. Incision wounds healed faster in olive oil treated group, and the group that was treated with methylated spirit and iodine than other treatment groups. This agrees with findings by El-Okdi *et al.,* (2008), who showed that cardiotonic steroids (Ouabain, Digoxin, Marinobufagenin and Tecinobufagenin) play an important *in vivo* wound healing effect in stimulating collagen synthesis by cardiac fibroblast signaling through the Na-K-ATPase pathway (Gupta, 2012). The findings equally agrees with the works of Schoner and Scheiner-Bobis (2007) showing skeletal muscle cell growth in rats With absence of scar formation, stimulated by both exogenous and endogenous cardiac glycoside, this was attributed to positive inotropic effect of bufadienodide

content of cardiac glycoside (Schonfield *et al.,* 1985; Botha *et al.,* 2007, Paddleton, 2010). In vivo quercetin release is also reported to have anti-inflammatory effect on injury (Monica *et al.,* 2005)

The element Zinc was found to be in higher in concentration in olive oil when compared to that in honey. Prassad (1995) reported that zinc is involved in protein synthesis and deficiency of this element in the body causes delay in wound healing (Heinemann, 1996), while high ingestion results in interference with copper absorption and indirectly interferes with wound healing (Marti and Allred, 1999). In this study, Zinc was found in optimum amount which is similar to daily requirement by a 4-8 year old human. Calcium plays an important role in homeostasis in mammalian skin and nerves and a modulator in keratocytes proliferation and differentiation in wound healing. Calcium is predominantly involved in factor IV in the homeostatic phase and in later stages of wound healing, plays a role in epidermal cell migration and regeneration patterns (Lansdowne, 2002), these may have accounted for early wound healing effect of olive oil and olive oil/honey combination treated groups when compared to other groups.

The rapid wound healing observed in olive oil and methylated spirit and iodine treated groups could be attributed to age of rabbits used , which is in agreement with similar findings by Ferguson and O'Kane (2004) who attributed rapid wound healing in rabbits to age (of the rabbits). The anti-inflammatory effect of terpenoids and cardiac glycosides (loggia, 2000; Monica *et al., 2005)* could result in low invasion of less differentiated inflammatory cells and low levels of TGF-ß 1&2 (tumor growth factor beta 1 and 2), platelets- derived growth factor and high levels of TGF-ß -3, hence, less scar formation observed. This could also be due to enhanced balanced anabolic and catabolic phases of wound healing process attributed to these phytochemicals (Wahl and Wahl, 1992, Zamboni *et al.,* 2005).

Iron plays a vital role in wound healing through enhanced red blood cell formation, oxygen transport and collagen production, though, this process involves other enzymatic activities in metabolic pathways (El-Okdi *et al.,* 2008).

The high concentration of Potassium in both olive oil and honey might have played a role in the wound regression effect observed in the groups treated with these

agents. It has been reported that potassium is a major intracytoplasmic ion required for proper membrane and nerve impulse functions and digestion (Reid *et al.,* 2005). It was reported to chemotactically, facilitate infiltration odf oxygen to the tissues and helps eliminate toxins from the body thereby hasten wound healing (James *et al.,* 1953).

Other elements such as Copper are involved in hemoglobin synthesis and serve as a cofactor in enzyme function while manganese is also a cofactor in some enzyme activities and involved in the synthesis of fatty acids, cholesterol and also involved in connective tissue development (Vishnoi, 1979, Kerstein, 1997). The presence of these elements in both honey and olive oil, though in trace quantities, might have played a role in the enhanced wound healing effects of these agents.

Conclusion

Honey and olive oil contain essential phytochemicals and mineral elements required by the body for enhanced and rapid wound healing. Wound treatment with olive oil produced less scar formation when compared with the synthetic drug-penicillin ointment and honey. Combination of honey and olive oil showed more enhanced wound healing effect than honey alone.

Recommendation

These natural products (honey and olive oil) can be used individually or in combination, and as a local content by pharmaceutical companies for the preparations of wound healing ointments and skin care products.

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