Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 6, 2015

Short Communication

ANTIBACTERIAL ACTIVITY OF OIL EXTRACT OF TRIGONA PROPOLIS

NIKEN PUJIRAHAYU[1], HALIMAHTUSSADDIYAH RITONGA[2], SATYA AGUSTINA L[1], ZAKIAH USLINAWATY[1]

[1]Department of Forestry, Faculty of Forestry and Environmental Sciences, [2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kampus Hijau Bumi Tridharma Anduonohu, Kendari, 93232, South East Sulawesi, Indonesia
Email: rahayuken08@gmail.com

Received: 04 Feb 2015 Revised and Accepted: 01 Mar 2015

ABSTRACT

Objective: This research is aimed to determine antibacterial activity of some solvents of raw propolis extracts of *Trigona sp* from Sulawesi, Indonesia and compare it with antibacterial activities of ethanolic and aqueous extracts.

Methods: Propolis samples were extracted with water, ethanol, propylene glycol, olive oil, and Virgin Coconut Oil (VCO). An agar-well diffusion assay was used to evaluate the antimicrobial potential of propolis against *Escherichia coli, Salmonella thypi,* and *Staphylococcus aureus.*

Results: The oily extract of propolis showed a potent antibacterial activity compared to the ethanol extracts against *Staphylococcus aureus* and *Esherichia coli*. Inhibition zone of Olive Oil Extracts of Propolis on *S. aureus* was higher (22.4 mm) than Ethanolic extracts and Water Extracts. Inhibition zone of Virgin Coconut Oil (VCO) propolis extract and Olive oil extract on *E. coli* were 9.5 mm and 9.3 mm, respectively. The oily extracts also showed higher action against *E. coli* compared with the ethanol extracts and propylene extracts.

Conclusion: The propolis extracts obtained with Virgin Coconut Oil (VCO) and olive oil as solvent have higher antibacterial activity than the ethanolic extracts. So the VCO and Olive Oil can be used to extract raw propolis.

Keywords: Antibacterial activity, Olive Oil Extracts of Propolis, VCO Extracts of Propolis, *Trigona sp.*

Propolis is a mixture of beeswax and resin which are collected by honey bee (*Trigona sp*) from plant buds, leaves, and exudates [1, 2]. Bee uses propolis not only as a building material for their hive but also a mean for maintaining a low level of bacterial and fungal consentration in the hive [3]. Specifically for *Trigona*, propolis is also used to construct storage pots for pollen and honey. Propolis is a soft and sticky substance when it is heated, and becoming hard and brittle when it is freeze.

Propolis consists of more than 300 different compounds including, flavonoids, phenolics, aldehydes lipophilic, flavonoid-aglycones and other compounds such as pollen, wax, vitamins, minerals and so on [4, 5]. Propolis has properties as bactericidal and fungicidal, antioxidant, antivirulant-inflammatory, [6, 7] and it is used as an alternative treatment for infections. A wide range influence of propolis on various microorganisms is as the result of combined activities of flavonoids and aromatic compounds [8].

Amongs natural products, propolis has received more attention due to its broad-spectrum antimicrobial activity against a wide range of pathogenic microorganisms. Propolis, also referred to "bee glue," is a generic name for resinous substance collected by honey bees *(Trigona sp)* from various plant sources [9]. The word propolis is derived from Greek as"pro" meaning "in defense of" and "polis" meaning "city," so it is referring to the defense of a city or a bee hive. Propolis is a strong, adhesive substance collected and used by bees for sealing holes in their honey combs and protecting their entrance from intruders [1, 10, 11].

Propolis contains variety of chemicals such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids and amino acids. The composition of Propolis depends on the species of honey bees and types of vegetation presenting in perspective geographic [12], and by the seasonal collection [13, 14]. Propolis that rich of bioflavonoid has antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory properties. Other properties of propolis, these are as a local anesthetic, reducing spasms, healing gastric ulcers, and strengthening capillaries. Propolis can be used by humans internally or externally [15, 12].

The extracting method of propolis used in biological assays may influence its activity. The common method is solid-liquid extraction, which uses ethanol in different concentration, methanol or water. The extract contains amino acids, flavonoids, terpenes, and cinnamic acid derivatives. It also contains lectin [16]. The extraction solvent influences the composition, and consequently its biological activities. The most widely used solvent for propolis preparation is aqueous ethanol, followed by such as ethyl ether, water, methanol and chloroform [17].

Another natural products that also as the top using treatment for a period time are the olive oil and the Virgin Coconut Oil (VCO). The usefulness of olive oil and VCO as herbals remedy is not in doubt. The benefits of olive oil are not only for treatment purposes but also as for food, cosmetics, and beauty to nourish skin's health. In this study, we used several kinds of oil (virgin coconut oil/VCO), olive oil, propylene glycol, water (aqueous) and ethanol as the solvent to prepare propolis extracts. The propolis oil extract presents more advantageous compared to being commonly used, ethanolic extract [17, 18]. Therefore, the objective of this study is to investigate the antibacterial activity of oil extract of Indonesian *Trigona* propolis on bacteria *Escherichia coli, Salmonella thypi,* and *Staphylococus aureus* and compare them with the ethanolic and aqueous extracts.

The chemicals were used in this study include: Ethanol (70%), potassium chloride (99.8%),peptones, potato extract, sodium chloride, potassium phosphate, and Na asetat were all purchased from Merck Co, Potato Dextrose Agar (PDA), Lurie Bertani (LB), gelatin, olive oil extract of propolis, Virgin Coconut Oil extract of propolis, ethanol extracts of propolis, aqueous extracts of propolis and propylene glycol extracts of propolis.

Raw propolis used for extraction was from Landono district, South Konawe, South East Sulawesi in 2013. The raw propolis was taken from the bee hives of *Trigona* that widely spread in Landono district. There were three strains of standard bacteria used in this study. They were provided by the Department of Microbiology, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, South East Sulawesi, Indonesia. They are *Escherichia coli, Salmonella thypi,* and *Staphylococcus aureus.*

The propolis extract (PE) was prepared according to a method presented by Matienzo dan Lamorena [19] and Al-Jumaily and Al-Obaida [20] with modifications [18]. Granulated propolis was

extracted with different solvents, these are water, ethanol, VCO, olive oil and propylene glycol (same concentrations) at 40 °C in shaker (Stuart GFL 1086). Thus, 25 g of propolis (finely grounded in a mixer) was extracted with 250 ml solvents (water, ethanol 70%, VCO, Olive oil and propylene glycol) at 40 °C into shaker in dark room for seven days. After that, the suspension was filtered (with Whatman filter paper No. 41), the residue was extracted again. Then for seven days the suspension was filtered every day. The yield of maceration was further dried in rotary evaporator (Laborota 4002) at 30 °C-40 °C. Then the dry extracts were weighed to gain the final extract. All the samples then were analyzed in triplicate.

The extracts were evaluated against the three bacteria: *Escherichia coli, Salmonella thypi,* and *Staphylococcus aureus* by utilizing agar diffusion method. The bacteria were grown on plates with Lure Bertani (LB) (30°C/15 days), then added 1 ml of sterile saline solution (0.85%) to prepare a spore suspension. The plates with LB (20 ml) were seeded by pour plate with 100 l of the spore suspension. Then 5 µl of the ethanolic and propolis oil extract were added in well (4 mm), with final volume of 100 µl for each well, completed with the respectively solvent, then the plates were incubated at 37°C for 24 h and the inhibition zone was measured with caliper. The inhibition zone width of growth of the tested microorganism was measured from the margin of the hole to its outer border. The value, stated in millimeters, is mean value of the three holes measured in one Petri dish. The assays were made in triplicate and ampicilline (50 µg/ml) were used as positive control, and each solvent plate were used as negative control [8, 17].

The data of the inhibition zone of propolis extract, which arestated in mean value, is the representative of three independent experiments. The means of different solvents were compared using analysis of variance (ANOVA) then continued by using Duncan's Multiple Range Test, $p<0.05$ was considered to be statistically significant.

The results of the study of the inhibitory effect of propolis extracts with various types of solvents on *E. coli, S. aureus* and *S. typhi* are shown in table 1. These results indicate that the type of solvent significantly affects on the inhibition zone. Propolis extract with olive oil and VCO as solvent have a greater inhibitory against *E. coli* and *S. aureus* and were significantly different compared to PG extract and ethanol extract of propolis, while the water solvent showed no inhibitory effect on the three bacteria that were observed. However, five of the propolis extract did not show any inhibitory effect against *S. typhi*.

Table 1: The mean of the diameters (mm)[1] of bacterial growth inhibited by different solvent of extract of Propolis on some Bacteria

Bacteria	Propolis Extracts					
	PG Propolis	VCO Propolis	Olive Oil-Propolis	Et-OH Propolis	Aquades Propolis	Ampicilline
E. coli	4.40±0.11^{c}	9.5±1.02^{b}	9.3±1.12^{b}	4.43±0.32^{c}	-	38.2±0.31^{a}
S. aureus	11.9±1.08^{c}	12.0±0.90^{c}	22.4±1.10^{b}	10.8±0.93^{c}	-	44.8±0.21^{a}
S. thypi	-	-	-	-	-	

[1]:Data are mean±standard deviation (n=3). Values within each row followed by different letters are significantly different at $P<0.05$

Table 1 shows that the Olive oil extract of propolis (OEP) and VCO extract of propolis (VEP) gives the largest zone of inhibition against the growth of *E. coli* and *S. aureus*t han Propylene glycol extract of propolis (PEP) and ethanol extract of propolis (EEP). However, the Aquades extract of propolis (AEP) did not show any inhibitory effects on the tested microorganisms. Not all extracts did effectively to *S. thypi*.

Based on previous studies, among the five of propolis extract that contain flavonoid, the sequence from the highest to the lowest is the extract from the PEP by 0.55%, 0.33 EEP, VEP of 0.25, 0.22 EAP and OEP 0.20 [18]. The result show that higher flavonoid content does not always have the ability to inhibit a greater growth of bacteria, although the presence of flavonoids substances is believed to be responsible for the ability of the antimicrobial activity of propolis [21, 22]. Antibacterial activity of propolis in addition to be affected by the flavonoids amount, it is also affected by the content of phenols in propolis [2, 6].

In this study, the greater ability to inhibit the growth of *E. coli* and *S. aureus* surprisingly indicated by the OEP and VEP, which have lower levels of flavonoids than PEP and EEP. This is an indication that there were other components that influence their effectiveness in inhibiting the growth of bacteria. Supposedly, components contain in the solvent Olive Oil and VCO adds the effectiveness of propolis extract toward its ability to inhibit any bacterial growth.

In propolis, flavonoid known as a substance to kill or inhibit many bacterial strains, inhibit viral enzymes, avoid free radicals, etc [23]. Significant correlation was found between the flavonoid content in propolis and MIC [24, 11]. The mechanism of flavonoids activity in inhibiting bacteria causing damage to the permeability of the bacterial cell wall, microsomes, and lysosomes because of interaction between flavonoids with bacterial DNA [25].

The mean diameters of microbial growth inhibited by different solvent of propolis extracts and standard ampicilline on *E. coli* are shown in table 1. Although inhibition zone of ampicilline was the highest, but VEP and OEP are more effective compared to EEP and PEP on *E. coli*. Propolis extracted by VCO had a stronger inhibitory effect on *E. coli* and it is similar to propolis extracted by olive oil. The greater inhibition zone of VEP allegedly is caused by some compounds of the solvent (VCO and Olive oil) which give the synergic effect to other compounds in propolis for an inhibitory effect of the extracts on bacteria growth.

The main compounds of VCO are monolaurin and lauric acid, that have antiviral, antibacterial and antiprotozoa activity [26]. Monolaurin is a non-ionic surfactant having two ends with different properties. One end is hydrophobic and the other is hydrophilic [27, 28]. Therefore, it can interfere the growth of bacteria, both gram-positive and gram-negative bacteria. Gram-negative bacteria outer membrane is *lipopolysaccharide* composed of lipids, polysaccharides and proteins, is more non-polar whereas gram-positive bacteria consists of a thicker peptidoglycan layer that has a polar in nature, so that they can be affected by monolaurin. Gram-negative bacteria have thicker lipo polysaccaride layers so that lauric acid and monolaurin can easily penetrate through the membrane, and its equally non-polar making pervasive, damaging the cell walls of bacteria, and the bacteria is died. When it against gram-positive, although the membrane lining layer contains a very small amount of fat, but because monolaurin is a surfactant so it can damage the bacterial cell membrane, causing membrane to lysis and then inhibited bacterial growth [28]. That was support our findings that propolis extracted by VCO had inhibitory effect both gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*).

Some researchers reported that Ethanol extract of propolis showed an antibacterial activity only toward the Gram-positive bacteria and fungi, whereas, there was no activity observed which against Gram-negative bacteria [2, 29]. However, it has been reported that EEP was effective on Gram-negative bacteria in higher concentrations [30]. This study also showed that EEP had inhibitory effect both on Gram-positive bacteria (*S. aureus*) and on Gram-negative bacteria (*E. coli*) even they were lower than ampicilline.

Other researchers from Spain reported that propolis extract in ethanol and propylene glycol in several different locations have the antimicrobial and antioxidant activity [38, 22]. They concluded that the extract is highly active against gram positive such as *Staphylococcus aureus, Streptococcus mutans, Candida albicans* and *Saccharomyces cerevisae*) and moderately active against *Streptococcus pyogenes* [39, 22]. The results of this study indicate that the inhibition zone of propylene glycol, VCO extract of propolis,

and ethanol extract of propolis is not significantly different in inhibiting *S. aureus,* but they are significantly different with Olive oil extract of propolis.

VEP and OEP also effective in inhibiting the growth of *S. aureus*, but OEP is more effective than other extracts inhibit *S. aureus.* EOP inhibition zone against *S. aureus* significantly different when compared with the EPG, EVP and EEP. Diameter of inhibition of EOP was22.4 mm, while the VEP by 12 mm, EEP 11.9 and PEP of 10.8 mm. Presumably, the magnitude of the inhibitory effect of OEP was because of the phenolic components contained in the Olive oil solvent. Several studies conducted on humans and animals both *in vivo* and *in vitro* showed that the phenolic components of olive oil has the effect of potentially biologically benefit resulting from antimicrobial activity, antioxidant and anti-inflammatory [31, 32].

Olive oil is composed of oleat acid, palmitat acid, sterol acids, simple phenols such hydroxtyrosol, tyrosol, catechol and other which have been considered responsible of the antimicrobial activity detected in the olive oil [33-35]. The most bactericidal polyphenols of olive oil were HyEDA and TyEDA, in particular the latter compound which is also known as oleocanthal [36, 37]. Our result showed that propolis extracted by olive oil had inhibitory effect to *S. aureus* and *E. coli* was the highest.

In Spain, some authors stating that ethanolic and propylene glycol extracts of propolis from different locations throughout the Basqu country had antimicrobial and antioxidant activity [38]. These authors reported the microbial activity of the same extracts of propolis which had antioxidant activity [39] and concluded that such samples were very active against Gram-positive bacteri and yeasts (*S. aureus, Streptococcus mutans, Candida albicans and Saccharomyces cerevisae*) and moderately active against Streptococcus pyogenes. They also found significant activity against the Gram-negative bacteria *Salmonella enterica*, whereas *E. coli* was resistan to propolis samples. The authors also detected a dose-dependent activity against the microorganisms tested and a strong correlation between total phenolic content and the antimicrobial activities and between flavonoids and antibacterial activity [22].

However, the oily extract was more potent on inhibiting the bacteria growth, promoted higher inhibition zone than that observed with the other extracts. In the antibacterial test, our data also showed that VCO extracts of propolis had similar inhibition zone with Ethanol and propylene glycol extracts against *S. aureus*. Moreover, the Olive Oil extract of propolis resulted in greater inhibition zone against *S. aureus* when compared to ethanol and propylene glycol extracts.

Very similar results for the antibacterial activity of propolis on *S. aureus*, were published by Sforcin *et al.* [30] and Silva *et al.* [17]. Our results showed that propolis extracted by Olive oil had a stronger inhibitory effect on *S. aureus* compared to propolis extracted by other solvents. These results are supported by a study of Medina *et al.* [37], that olive oil exerted a strong bactericidal action against a broad spectrum of microorganisms, This effect is generally significant against Gram-positive bacteria as compared to Gram-negative bacteria. Thus, olive oil showed that bactericidal activity is not only against potential harmful bacteria of the intestinalmicrobiota (*Clostridium perfringens* and *E. coli*), but also it is against beneficial microorganisms such as *Lactbacillus acidophilus* and *Bifidobacterium bifidum*. Otherwise, most of the food borne pathogens tested (*Listeria monocytogenes, Staphylococcus aureus, Salmonella enteritidis*, *Enterica, yersinia s,* and *Shigella sonnei*) did not survive after one hour contact with olive oils.

The advantage of use the solvents VCO and Olive Oil is, they are both foodstuffs, not an antibiotics, which can be directly used with propolis without having to be remove it. VCO and Olive Oil can enhance the antimicrobial activity of propolis if they are used a solvent to extract propolis. These results also suggest that the antimicrobial effects of propolis vary for different solvent and microorganism species, this accordance with study by Agarwal *et al.* [11] and Ivancajic *et al.* [8].

Propolis demonstrates antibacterial activity on Gram–negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* when extracted by VCO, Olive Oil, Propylene glycol and ethanol. However, all the extracts is not effective to *S. thypi*. Propolis extracted in Olive oil has the most inhibitory activity on *S. aureus*, while propolis extracted in VCO has the most intensive antibacterial activity on *E. coli*. *S. thypi* is relatively insensitive to the activity of propolis. The benefits of use the solvents VCO and Olive, is that they are the edible solvent, so that we can use them directly without having to remove it. In addition, these oil can enhance the inhibitory effect of propolis against gram-negative bacteria such as *E. coli*. Next study is recommended to determine the biologically active compounds of VCO extracts of propolis and Olive oil extracts of propolis and its antimicrobial activity to other species.

ACKNOWLEDGMENTS

Researchers would like to thank to Ditlitabmas Dikti and Research Institute of Universitas Halu Oleo for providing funding of BOPTN Research Grant program in 2014.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Ghisalberti EL. Propolis A review. Bee World 1979;60:59-84.
2. Yaghoubi SMJ, Ghorbani GR, Soleimanian ZS, Satari R. Antimicrobial activity of Iranian propolis and its chemical composition. DARU 2007;15(1):45-8.
3. Popova M, Silici S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomed 2005;12:221-8.
4. Marcucci MC, Ferreres F, Gracia-Vigueraa C, Bankova VS, De Castro SL, Danas AP *et al.* Phenolic compounds from Brazalian propolis with Pharmacological activities. J Ethnopharmacol 2001;74:105-12.
5. Greenaway W, Scaysbrook T, Whatley FR. The analysis of bud exudates of populus X euramericina and of propolis, by Gas Chromatography-Mass Spectrometry. Proc Royal Soc London 1987;232:249-72.
6. Nallahalli SS, Bhushanam M, Ravikumar H. Antimicrobial activity of propolis of *trigona sp* and *apis mellifera* of karnataka, India. Prime J Microb Res 2012;2(2):80-5.
7. Kosalec I, Pepeljnjak S, Bakmaz M, Vladimir‐Knezević S. Flavonoid analysis and antimicrobial activity of commercially available propolis products. Acta Pharm 2005;55:423-30.
8. Ivancajic S, Mileusnic I, Cenic-Milosevic D. In vitro antibacterial activity of propolis extracts on 12 different bacteria in condition of 3 various pH values. Arch Biol Sci 2010;62(4):915-34.
9. Burdock GA. Review of the biological properties and toxicity of bee propolis(propolis). Food Chem Toxicol 1998;36(4):347-63.
10. Santos FA, Bastos EM, Uzeda M, Cavahlo MA, Farias LM, Moreira ES, *et al.* Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. J Ethnopharmacol 2002;80:1-7.
11. Agarwal G, Vemanaradhya GG, Mehta DS. Evaluation of chemical composition and efficacy of Chinese propolis extract on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*: An *in vitro* study. Contemp Clin Dent 2012;3(3):256‐61.
12. Selvan AK, Prabhu T. Extraction of propolis from beehives and characterization of its constituens and medicinal properties: a review. Int J Adv Eng Tech 2010;1(3):50-3.
13. Kalogeropoulos N, Konteles SJ, Troullidou E, Mourtzinos I, Karathanos VT. Chemical Composition, Antioxidant activity and antimicrobial properties of propolis extracts from greece and cyprus. Food Chem 2009;116:452–61.
14. Tylkowski B, Trusheva B, Bankova V, Giamberini M, Georgi P, Nikolova A. Extraction of biologically active compounds from propolis and concentration of Extract by nanofiltration. J Membr Sci 2010;348:124–30.
15. Barros MP, Souza JP, Bastos JK, Andrarde S. Effect of Brazilian green propolis on experimental gastric ulcers in rats. J Ethnopharm 2007;110:567–71.
16. Coneac G, Gatifanu E, Hadaruga DI, Hadaruga NG, Pinzaru IA, Bandur G, *et al.* Flavonoid content of propolis from the west side of romania and correlation with the antioxidant activity. Chem Bull Politechnic 2008;53(67):56-60.

17. Silva KR, Francielle TM, Kelly AD, Shierly DK, Herta SDS. Antimicrobial activity from a Brazilian propolis oily extract compared with other propolis extract. Revista Ciences Eatase Naturis 2010;12(2):327-38.

18. Pujirahayu N, Ritonga H, Uslinawati Z. Properties and Flavonoid content in propolis of some extraction method of raw propolis. Int J Pharm Pharm Sci 2014;6(6):338-40.

19. Matienzo Acc, Lamorena. Extraction and Initial characterization of Propolis From stingless Bee (*Trigona biroifriese*). In: Proceeding of The 7th. Asian apicultural association conference and 10th BEENET Symposium and Technofora; 2004. p. 321-29.

20. Al-Jumaily EF, Al-Obaida RS. Extraction and purification of flavonoid from iraqi propolis (Bee Glue) and evaluate its antioxidant activity. Int Interdisciplinary Res J 2013;3(5):9-17.

21. Marcucci MC. Propolis: Chemical Composition, Biological Properties and Therapeutic Activity. Apidologie; 1995. p. 83-99.

22. Miguel MG. Chemical and biological properties of propolis from the western countries. Int J Pharm Pharm Sci 2013;5(3):403-9.

23. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther 2002;96:67-202.

24. Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini AG, *et al.* Validated methods for the quantification of biologically active constituents of poplar‐typepropolis. Phytochem Anal 2004;15:235-400.

25. Sabir A. Dental Pulp Inflammation Response in Mice After Application of Propolis Ethanol Extract (EEP). Dental J 2005;38(2):77-83.

26. Ugbogu OC, Onyeagba RA, Dan Chigbu OA. Lauric acid content and inhibitory effect of palm kernel oil on two bacterial isolated and *Candida albicans*. Afr J Biotech 2006;5(11):1045-7.

27. Widiyarti G, Hanafi M, dan Suwarso WP. Study on the synthesis of monolaurin as antibacterial agent against *Staphylococcus aureus*. Indo J Chem 2009;9(1):99-106.

28. Loung FS, Silalahi J, Suryanto D. Antibacterial activity of Enzymatic hydrolyzed of virgin coconut oil and palm kernel oil against *Staphylococcus aureus, Salmonella thypi* and *Escherichia coli*. Int J Pharm Tech Res 2014;6(2):628-33.

29. Lu LC, Chen YW, Chou CC. Antibacterial and DPPH free radical-scavenging activities of the ethanol extract of propolis collected in Taiwan. J Food Drug Anal 2003;11:277-82.

30. Sforcin JM, Fernandes JA, Lopes CAM, Bankova V, Funari SRC. Seasonal effect on Brazilian propolis antibacterial activity. J Ethnopharmacol 2000;73:243-9.

31. Ghazghazi H, Chedia A, Safa H, Wissem M. Antibacterial, Antifungal and antioxidant activities of Tunisian *Olea europaea* ssp oleaster fruit pulps and its essenstial fatty acids. Int J Pharm Pharm Sci 2015;7(1):52-5.

32. Paudel S, Thakur M. Lamichhane JR. Antimicrobial activity of wild olive crude extracts *in vitro*. Int J Pharm Sci Res 2011;2(3):110-3.

33. Della Greca M, Monaco P, Pinto G, Pollio A, Previtera L, Temussi F. Phytotoxicity of low-molecular-weight phenols from olive mill waste waters. Bull Environ Contam Toxicol 2001;67:352-9.

34. Keceli T, Robinson RK. Antimicrobial activity of phenolic extracts from virgin olive oil. Milchwissenschaft 2002;57:436-40.

35. Brenes M, García A, de los Santos B, Medina E, Romero C, de Castro A, Romero F. Olive glutaraldehyde-like compounds against plant pathogenic bacteria and fungi. Food Chem 2011;125:1262-6.

36. Beauchamp GK, Keast RS, Morel D, Lin J, Pika J, Han Q, *et al.* Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil. Nat 2005;437:45-6.

37. Medina E, de Castro A, Romero C, Brenes M. Comparison of the concentration of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. J Agric Food Chem 2006;54:4954-61.

38. Bonheví JS, Gutiérrez AL. The antimicrobial effects of propolis collected in different regions in the basque country (Northern Spain). W J Microb Biotechnol 2012;28:1351-8.

39. Bosio K, Avanzini C, d'Avolio A, Ozino O, Savoia D. *In vitro* activity of propolis against *Streptococcus pyogenes*. Lett Appl Microbiol 2000;31:174-7.