THE EVALUATION OF HOT AND MICROWAVE EXTRACTIONS OF *Ziziphus vulgaris* (L.) LEAVESAND STUDY THEIR EFFECTS ON WOUNDS HEALING

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ABSTRACT

The present study was concerned with comparison of hot extract with microwave extract and the pharmacological potency of *Zizyphusvulgaris* (L.) leaves extract towards wound healing effect in mice. The study include the use of three groups of wounds in order to indicate the effect of extracts in wound healing. Two extracts were used the first were boiling extracts and the second microwave extracts and control group. Many tests used to indicate the components of these extract (tannic acid test, saponine test, drgendroff test) and TLC analysis. The results of this research were improving the wounds healing activity of *Ziziphus vulgaris*. The hot extract was more active than microwave extract as wounds healer. The therapeutic role of *Ziziphus vulgaris* leaves extract in wound healing, mostly due to synergism of presence of alkaloids, carbohydrates and flavonoids.

INTRODUCTION

Afford ability and accessibility of the medicinal plants have made them as an important part of many people's life all over the world. The medicinal plants selection is a conscious process, which has led to an enormous number of medicinal plants being consume in many cultures in the world (1). According to the World Health Organization (WHO), due to the poverty and lack of access to modern medicines, about 65-80% of the world's population in developing countries depends on plants for their primary health care (2, 3). It is estimated that close to 25% of the active compounds, in currently prescribed synthetic drugs were first identified in natural sources especially in plants(4).

The *Ziziphus vulgaris*(L.) commonly known as Jujubeis a deciduous tree, native to the warm-temperate and subtropical regions including North Africa, South Europe,

Mediterranean, South and East of Asia and Middle East. It belongs to (Rhamnaceae: family)(5).The fruits (fresh or dried) are used in traditional medicine to treat diabetes, colds and coughs (6, 7). These fruits are also use in Chinese and Korean traditional medicine, where they are believe to alleviate stress (8). From currently available pharmaceutical studies, additional pharmaceutical applications of *Z. vulgaris* include antifungal, antibacterial, antiulcer, anti-inflammatory, sedative and antiseptic that makes them very effective in wound healing (9, 10).

Wounds are physical injuries that result in an opening or break of the skin. Wound healing process holds several steps, which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodelling of connective tissue, collagenisation and acquisition of wound strength (11). Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (12). The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink or white (13).

The antioxidant is defined as a substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (14, 15). Antioxidants are of interest to biochemist because they help to protect the human body against injuries induced by reactive free radicals generated in atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and even in aging process (16, 17).There are much evidence on the efficient anti-oxidative activity of natural products and their derivatives, thereby, their association with anti-cancer, hypolipidemic, anti-aging and anti-inflammatory activities (15-18). Anti-oxidative capabilities of *Z. Vulgaris* were evaluate by determining its effect on cell membrane of hepatocyte and red blood cell haemolysis.

A survey of the literature revealed that a number of cyclopeptide,isoquinoline alkaloids, flavonoids, terpenoids and their glycosides have found in various amounts in most Ziziphus species. The leaves of these plants contain betulic and ceanothic acids, various flavonoids, saponins, erols, and triterpenes. In *Ziziphusvulgaris,* there are 13 types of saponins, figure 1 shown some of these compounds.

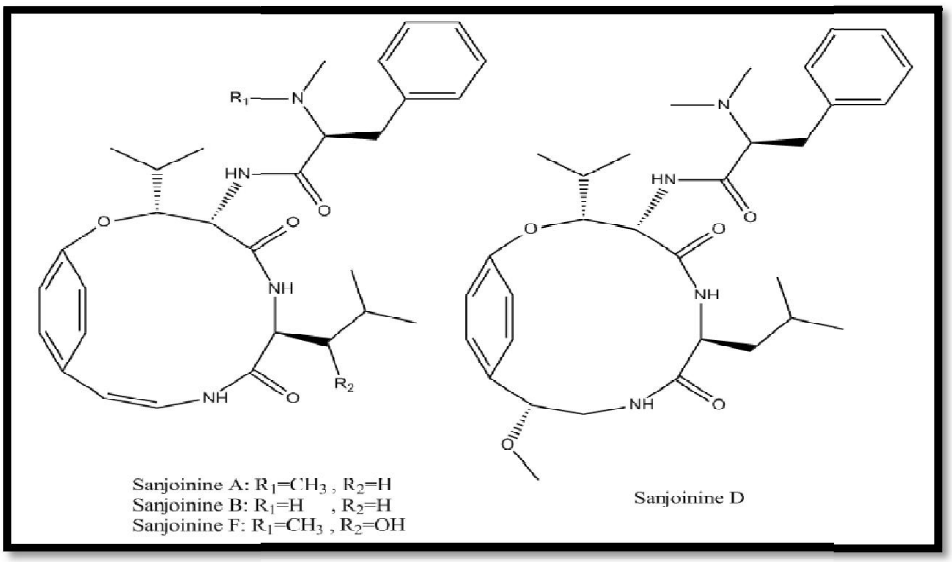

Figure 1: Structure of SanjoinineA, B, F and D.

MATERIALS AND METHODS

The plant was obtained and collected from Basrah (south of Iraq), in period of March to April in 2015.The plant materials were dried. After complete drying, the materials were crushed and grinded to form coarse powder.

Extraction in hot water

The first extract was prepared by using boiling process as following; the dried powders of plant material (30g) added to 300 ml of distilled water. The mixture was reflex for 3 hours for extraction. The mixture cooled to room temperature, then filtered. The filtrate were concentrated to quarter volume in rotary evaporator, then transport to Petri dish and left at room temperature to dryness (19).

Extraction in microwave

The second extract was prepared by extraction in microwave oven. Leaves powder 30g was suspend with 300ml of distilled water, the mixture were placed in microwave oven for 10min. The mixture cooled to room temperature, then filtered. The filtrate were concentrated to quarter volume, then transport to Petri dish and left at room temperature to dryness, and then the dried powder was collect.

Preparation of suspension

To use this product 0.75mg was dissolve in 1ml of distilled water with gentle mixing, and applied on the wound one time a day.

Experimental animals

Three groups of mice were used each group involves sixmice in the same age(3 months) and weight (18-20g). They were individually housed, maintained under standard environmental conditions of temperature 25 ± 2 ^{o}C in animals' house. Their foods were diet filled with proteins and carbohydrates. They were drink tap water.

The Wound Healing Evaluation

Screenings for the wound healing activity were performed by excision wound model. The animals were divided into 3 groups of 6 animals in each. Group-1: untreated, as the control; Group-2: the wound was in the right side (use ointment prepared with boiling extract); Group-3: the wound was in the left side (use ointment prepared with microwave extract).

Excision Wound Model

The animals were depilated on the vertebral area prior to creation of wounds and a predetermined diameter of 8-10 mm in skin in its full thickness was excise under ether anaesthesia. The suspension was applied once every 3 day for 14 days. Wounds were left undressed to the open environment and the animals were kept individually in same cage(20, 21). The wounds diameters were measured by using electronic digital micrometer apparatus.

RESULTS

Tests for extractions compositions

Tannic acid test was used to prove presence of alkaloid and protein, the result was positive (yellow precipitation occurs).Dragen droff test was used to prove presence of alkaloids, the result was positive, (pale precipitation). Alkaline addition was used to prove presence of flavonoids, the result was positive, (yellow colour appeared).Saponine test, the solution of extract form foam, which was stable for at least 2min, which indicate presence of saponine compounds (22).

Measurement of pH by using pH meter, the test was prepared by dissolving 0.2 g in 30ml of distilled water. The pH value for boiling extract was 6.66 at 27.9 ^{o}C .and for microwave extract was 7.74 at 27.9 ^{o}C.

Thin Layer Chromatography (TLC) analysis

The point A refers to boiling extraction, while point B refers to microwave extraction. The first TLC was made by using hexane: ethyl acetate 1:1 (22), the result was show in Table 1.

Table 1. The $R_f$value of the spots for both extracts.

	Solvent system	The $R_f$value of the spots	
		Spot A	Spot B
1	Hexane:EtAc 1:1	0.106 0.15 0.25	0.106
2	Hexane:THF 1:1	0.07 0.34 0.48 0.87	0.21 0.28
3	Hexane:EtAc:Ethanol 1:1:0.5	0.05 0.15 0.20 0.28 0.97	0.02 0.12 0.17 0.74 0.97

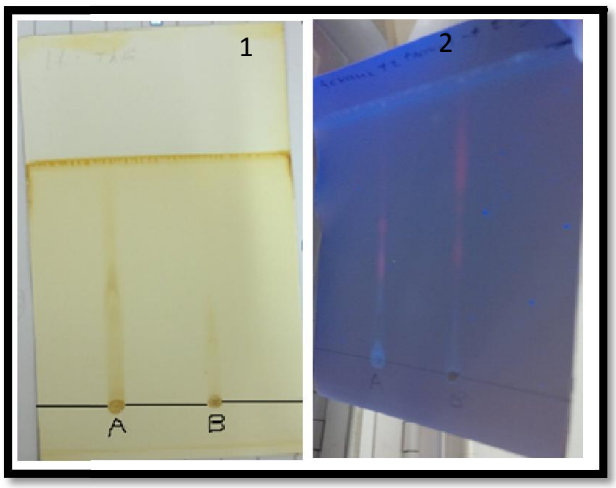

Figure 2: The pictures of TLC; 1.Inhexane:EtAc (1:1) developed by iodine in day light, 2. In Hexane:EtAc: Ethanol (1:1:0.5) in ultraviolet light.

Wound healing effects

Table 2 shown the diameter in millimetres (mm) of wounds over time of experiment. The statistical analysis show the boiling extract was more significant ($p<0.01$) than microwave extract to healing the wounds.

Percent of wound healing were calculated as in equation below (23):

$$\text{The percent of healing} = \frac{healed\ area}{total\ wound\ area}\ x100$$

The results of the percentage of healing shown in table 3 and figure 3 show the graph of diameter reducing of wound through experimental time.

Table 2. The diameter of wounds at different experimental time.

Groups	Diameter of wounds in (mm)				
	Zero day	2 day	8 day	10 day	13 day
Control group	10±1.3	8.05±0.8	6.45±0.5	4.91±0.2	4.33±0.3
Boiling extraction	9.3±1.1	6.9±0.6*	4.1±0.9*	1.44±0.4**	0.35±0.1**
Microwave extraction	9.6±0.7	9.07±1.2	5.92±0.6	3.87±0.7*	2.85±0.2*

NOTE: The values above are the mean value ± standard error(SE).
*=p<0.05, **=p<0.01 according to ANOVA t-test.

Table 3. Percentages of wounds healing.

Groups	The percentage of wound healing			
	2 day	8 day	10 day	13 day
Control group	10.5%	28.3%	45.4%	51.8%
Boiling extraction	23.3%	54.4%	84%	96%
Microwave extraction	9.3%	40.8%	61%	71.5%

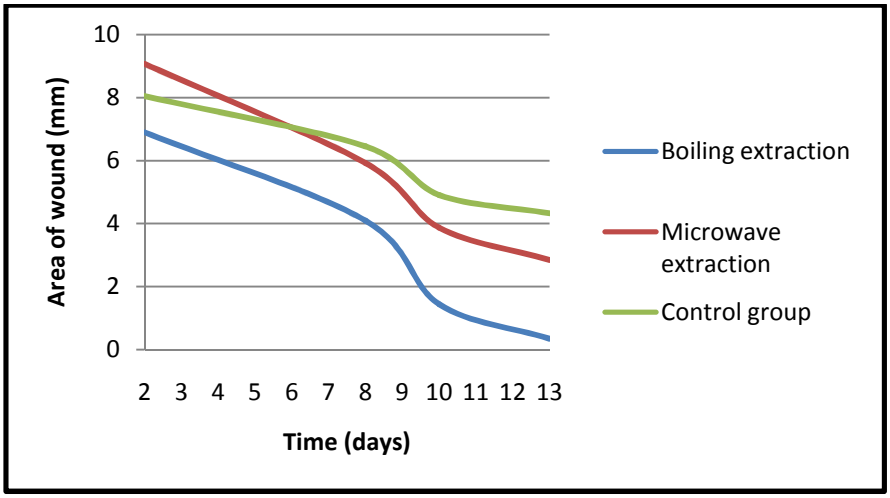

Figure 3: Diameter of wound remaining with time in (days).

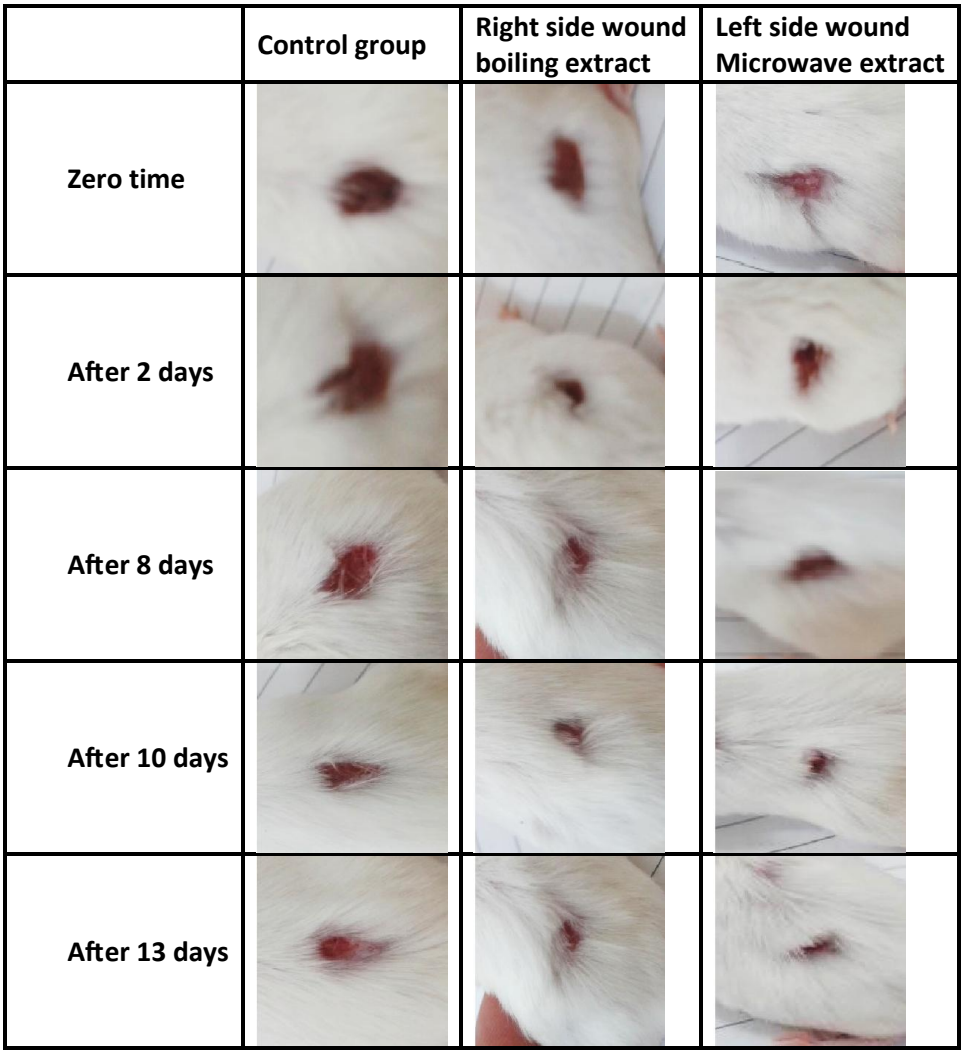

Figure 4: Area of wounds with time in (days).

DISSCUSSION

According to the results, Extraction by boiling (hot water) was better yield more quantity 2g than microwave extraction 0.50g. In wound healing effect, boiling extract was more effective and have good healed properties high significant ($p<0.01$) than microwave extract, because boiling extract yield more carbohydrates and lipid that are needed in wound healing (more building). Regarding to the mechanism for wound healing. Pentose phosphate pathway will be stimulated by production of ribose and NADPH, in which ribose act to manufacture of DNA and RNA while NADPH use for synthesis of lipid that use in synthesis of cell membrane, carbohydrates like glucose can provide energy for anabolism proses and can convert to ribose in pentose phosphate pathway (24). The boiling extract has slightly acidic pH 6.66 while microwave extract slightly basic pH 7.74 this indicated a different compounds were

extracted, this may be make boiling extract more effective than microwave extract. The test indicate that both extract have the same component (alkaloid, flavonoid, saponins and protein) while TLC analysis indicate presence of different component according to the different spot that occurs (different in shape and size). Both extract has been showing zero percent of contamination because Zizphus extract show anti septic activity.

CONCLUSION

Zizphus vulgarise is very important plant that has wide benefits in medical field. It showed effective properties in wound healing that belong to its component (alkaloid, flavonoid and protein) that facilitate the healing process. The boiling extract was more effective and potent than microwave extract in wound healing compared with control group. We use different methods for extraction (boiling and microwave) at same solvent (water) and we found that there are different chemical constituents in both extract depend on methods of extraction.

تقدير المستخلص الحار ومستخلص المايكرويف لأوراق نبات السدر *Ziziphus vulgaris*(L.) ودراسة تأثيرهما على شفاء الجروح

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الخلاصة

تركزت الدراسة الحالية على المقارنة بين الاستخلاص الحار والاستخلاص بالمايكرويف وقابلية المستخلصان على شفاء الجروح في الفئران المختبرية. استخدمت عدة كشوفات للكشف عن المركبات الموجودة في كلا المستخلصين مثل كشف حامض التانيك وكشف الصابونين وكشف دراكندروف وتقنية كروماتوغرافي الطبقة الرقيقة. كما تضمنت الدراسة استعمال ثلاث مجاميع من الجروح لبيان مدى تأثير المستخلصان على شفاء الجروح، المجموعة الأولى كانت للمستخلص الحار والمجموعة الثانية لمستخلص المايكرويف ومجموعة السيطرة. اثبتت النتائج ان مستخلص نبات السدر له قابلية على تسريع شفاء الجروح. وان المستخلص الحار أكثر فعالية من مستخلص المايكرويف. يعود التأثير العلاجي لمستخلص أوراق نبات السدر لتعاون وجود القلويدات والكربوهيدرات والفلافونيدات.

REFERENCE

1. Heinrich M., Barnes J., Gibbons S. and Williamson E.M.(2004). Fundamentals of pharmacognosy and phytotherapy. Elsevier science Ltd.Oxford.

2. Calixto J.B.(2005). Twenty-five years of research on medicinal plants in Latin America: A personal view. *J. Ethnopharmacol*. 100, 131-134.
3. Steiner R.(1986). Folk medicine. The art and the science. *American Chemical Society*, Washington DC, 92-97.
4. Halerstein R.A.(2005). Medicinal plants: Historical and cross-cultural usage patterns. *Ann. Epidemiol*. 15, 686-699.
5. SalehiSurmaghi M.H.(2010). Medicinal plants and phytotherapy. 3rd ed. Vol. 3, DonyaeTaghzieh Publications., Tehran.
6. Han B.H. and Park M.H.(1987). Alkaloids are the sedative principles of the seeds of Zizyphus vulgaris var. spinosus. *Arch Pharm Res*., 10, 203-207.
7. Han B.H., Park M.H. and Han Y.(1990). Cyclic peptide and peptide alkaloids from seeds of Zizyphus vulgaris. *Phytochem*. 29, 3315-3319.
8. Mill G.P.(2009). Demonstration of the psychotropic effect of mother tincture of Ziziphusjujuba. *Phytother*. 7(1), 31-36.
9. Mahajan R.T. andChopda M.Z.(2009).Phyto-pharmacology of Ziziphusjujuba Mill. - A plant review. *Pharmcog Rev*. 3(6), 320-329.
10. Jiang J.G., Huang X.J., Chen J. and Lin Q.S.(2007). Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen Ziziphusjujuba. *Nat Prod Res*. 21(4), 310-320.
11. Reddy J., Suresh P., Rajeswara R. and Reddy M.S.(2002). Wound healing effects of HeliotropiumIndicum, plumbagoZeylanicum and AcalyphaIndica in rats. *J Ethnopharmacol*. 79, 249-251.
12. Begum D. andNath S.C.(2000).Ethnobotanical review of medicinal plants used for skin diseases and related problems in northeastern India. *Journal of Herbs, Spices and Medicinal Plants*. 7(3),55-93.
13. Thakare V.M., Chaudhari R.Y. andPatil V.R.(2011). Wound Healing Evaluation of Some Herbal Formulations Containing Curcuma Longa and CynodonDactylon Extract.*International Journal of Phytomedicine*. 3,325-332.

14. Rhee M.H., Park H.J. and Cho J.Y.(2009). Salicorniaherbacea: Botanical, Chemical and pharmacological review of halophyte marsh plant. *J. Med. Plants Res*. 3(8), 548-555.
15. Mates J.M., Perez-Gomez C. and Nunez de Castro I.(1999). Antioxidant enzymes and human diseases. *Clin. Biochem*. 32, 595-603.
16. Aruoma O.I.(2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat. Res*. 523, 9-20.
17. Hemati A.,Azarnia M. andAngaji A.H.(2010). Medicinal effects of *Heracleumpersicum*(Golpar). *Middle-East J. Sci. Res*. 5(3), 174-176.
18. Cho J.Y., Prak S.C., Kim T.W., Kim K.S., Song J.C., Kim S.K., Lee, H.M., Sung H.J., Park H.J., Song Y.B., Yoo E.S., Lee C.H. and Rhee M.H.(2006). Radical scavenging and anti-inflammatory activity of extracts from *Opuntiahumifusa*.Raf. *J. Pharm. Pharmacol*. 58, 113-119.
19. Ramadhan U.H. (2012). The anti-nociceptive effect of *Cassia acutifolia* (Delil) leaves extracts on mice. *Basrah Journal of Veterinary research*. 11(3), 52-58.
20. Farahpour M.R. andHabibi M. (2012). Evaluation of the wound healing activity of an ethanolicextract of Ceylon cinnamon in mice. *VeterinarniMedicina*. 57(1), 53-57.
21. Wosgrau A.C.C., Jeremias T.S., Leonardi D.F., Pereima M.J., Di Giunta G.D. andTrentin A.G.(2015). Comparative Experimental Study of Wound Healing in Mice: Pelnac versus Integra.*PLOS ONE*. 10(3), e0120322. doi:10.1371/journal.pone.0120322.
22. Reich E. and Schibli A. (2007). High performance thin layer chromatography for the analysis of medicinal plants. Thieme medical publishers Inc. New York.
23. Ramadhan U.H., Hussein N.A. and Abood H.S. (2012). Antimicrobial Photodynamic Therapy with Single Application of Phthalocyanines in *Staphylococcus aureus* and *Escherichia coli* Wounds Infections. *International Journal of Pharmaceutical Sciences and Nanotechnology*. 5 (3), 1785-1789.
24. Horton H.R., Moran L.A., Scrimgeour K.G., Perry M.D. and Rawn J.D.(2006). Principles of Biochemistry. Fourth edition. Pearson education, Inc. New Jersey.