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The Bio Adhesive Property of Some *Saudi* Herbal Plants Extracts in Wound Healing in Rats

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Abstract

In the current study, we investigated the active ingerdeints and potential of methanol extracts of Walnut leaves (*WL*), Flaxseed (*FL*) Chamomile (*CH*), Arnica (*AR*) , Sage *(SG)* and Evening primrose (*EP*). *as antioxidant, antibacterial and wound healing effeacy in rats.* In current study, the highest inhibitory effect of IC50 against protein denaturation was detected for ER. The average of zones of inhibition of SG and EP with zone 20 at 200mg/0.5ml had very high significant differences compared to positive control whereas they do not have inhibition zones at concentration 5mg/0.5ml. The WL, FL, CH and AR have no effects against MRSA. Data obtained showed that, a significant decreased in the elevels of hydroxyproline ($p<0.001$) content, reduced glutathione ($p<0.01$) , mylopeoxidase MPO ($p<0.01$) and EGF-β ($p<0.001$) in diabetic rats versus contral. Diabetic rats treated with either extarcts of *WL*, *FL*, *CH*, *SA*, *AR*, *ER* showed improvement in these parameters compared with untreated ($p<0.001$). But the ER is the most potent than other extract. It was concluded that Sage (*SG*) showed antimicrobial against MERSA at high concentartions compared with other extarcts. The WL demonstrated the maximum one. While Evening primrose showed potent inflammatory effect and potent wound healing in diabetic rats.

Keywords: Anti-inflammatory; Antioxidant; wound healing; herbal Saudi.

1. Introduction

Herbal medicine researchs have attention for effective improving health and good nutritional status because of the presence of bioactive compounds and antioxidant potency . For that, it was applied in the treatment of numerous diseases rise with drug development [1]. Up to 30 % of medications are extracted or originated from the herbal medicine [2-5]. Saudi Arabia charaecterized in Arabian region with valuable flora medicinal plants [6].

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Antioxidants are any substance significantly delays or prevent oxidative damage in target molecule. Moreover, antioxidants prevent many diseases through reduced free radicals or scavenging reactive oxygen species. The antioxidant capability of plant foods has been taken as a marker of their beneficial outcomes on human health Additionally, the main cause of the chronic disease is reactive oxygen species.The primary feature of an antioxidant is it capability to trap free radicals. Antioxidants act as a defensive medicine for several diseases by reducing the generation of free radicals or scavenging reactive oxygen species The antioxidant capability of plant foods has been shown as an indicator of their useful effects on human health.In broad biological terms, an antioxidant can be defined as any substance that inhibits or delays oxidative damage to a target molecule [7].

Inflammation is a biologic response in tissues when harmful stimuli attack, including impaired cells, pathogens, injury and irritants [8]. Extraction is the key procedure through which biomass materials which produce bioactive components [9]. The extraction process's goal is to improve the quantity of the target compound and maximize its biological activity [10]. Different factors affect extraction yields and biological activity such as extraction techniques and solvents [10]. Several organic solvents involving methanol, n-hexane and chloroform, have been used to extract bioactive components from plant material can identified [11]. In view of the fact of the diversity of bioactive components and their differing solubility characteristics in diverse solvents, the optimum solvent for extraction of the plant depends on the compound to be separated and the composition of the individual plant [12]. Consequently, recommending a suitable extraction solvent for individual plant materials is commonly tricky. In order to contribute to the knowledge of plants from Saudi Arabia. This study aimed to invrstiagte the bioadhesive paroperty of four types of extracts including [Walnut leaves (*Juglans regia*), Flaxseed (*Linum usitatissimum*) Chamomile (*Matricaria chamomilla*), Arnica (*Arnica montana*), Sage (*Salvia officinalis*) and Evening primrose (*Oenothera biennis*)] as antioxidant , antiinflammtory , antibacterial and accelarte wound healing.

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2- *Methodology.*

2.1Sample Preparation.

Arial parts of different plants were collected from different Al-Taif region, *Saudi Arabia*. Samples wer identified by staff members at Botany Dep, Faculty of Science, King abdulaziz University. Walnut leaves (*WL*), Flaxseed (*FL*) Chamomile (*CH*), Arnica (*AR*) , Sage *(SG)* and Evening primrose (*EP*). Plants cleaned, ground and mashed into powder using grinder machine (Al Saif, SA) and put in vacuum bags then stored in well-closed container.

In the current study, we investigated the active ingerdeints and potential of methanol extracts of Walnut leaves (*WL*), Flaxseed (*FL*) Chamomile (*CH*), Arnica (*AR*) , Sage *(SG)* and Evening primrose (*EP*). *as antioxidant, antibacterial and wound healing effeacy in rats.*

2.2.Preparation of different extracts.

Plant sample (100g) extracted with pure methanol (1:2 weight volume) in water bath at 60°C for 24 houre. The subsequent extract was filtered through (Whatman No.1). Obtained extracts dried by removing its solvent under the vacuum of the rotavapor evaporator.

2.3.DPPH Free Radical Scavenging Assay.

The concentration of plant extracts starts from 500 µg/mL. One mL of each dilution was mix up with 1 mL of (0.004%) DPPH solution and incubated at 37°C for 30 min. The absorbance of mixture was evaluated at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control.

2.4.Anti-inflammatory Activity.

In order to estimate the anti inflammatory activity of plants extracts influence by organic solvents, the following method of protein denaturation was used. Briefly, serial dilution of each solvent by dimethyl sulfoxide (DMSO). The concentration of plant extracts starts from 500 µg/mL. The

reaction starts by adding 1 mL of bovine albumin with 1 mL of plant extracts, incubated at 37°C for 20 min. Then, it is heated 30 minutes to 51°C then let it cooled and read using a spectrophotometer at 660 nm. Aspirin was applied for comparison as a positive control, while DMSO was worked as a negative control. Measurements were taken in triplicate.

2.*5.Antimocrobial activity of different extarcts against* methicillin resistant Staphylococcus aureus MERSA

Gram positive MRSA isolate was identified by Microbiology Dep KAU hospital, Jeddah. Using well diffusion method NCCLS (14).

2.6.Experimental design in vivo study.

Handling with aniamls and experiment were done according to ethical commettee of king abdulaziz University, Jeddah, Saudi Arabia.Twenty male albino rats weighing (150-180g) were obtained from animal house, KFMRC (Jeddah) and adapted for one week before experiment in animal house,at 22^{o}C .

Rats were divided randomly to two major experimental groups. Group I (5 rats) served as control. GroupII (15 rats) :Diabetes rats were induced by *I.P.* injection of 65 mg/kg b.w of streptozosin. Diabetic was developed after 24 hours, if blood glucose was >270mg/dl .

Dabetic rats were anesthetized with thiopental and part of their back hair skin was shaved with blade and 2*x*1cm in diameter skin was removed using sterile sissor and forceps.

The diabetic animals with injury were divided into 7 subgroups (each 3 rats) as following:(a) rats received dressing with 5% extarcts in sterile water of (WL, FL, CH and AR , SG and EP) respectively and positive control group 5% fucidine creame. The application was contioue for 2 weeks. Based on a three stage healing process for evaluation of final process of the wounds and its scoring (9-10).

At the end of experiment, blood samples were collected for assay of reduced gltathone (GSH), collagen, myloperoxidase enzyme and TGF-β using kit from Biodiagnaostic Company.

2.7. Statistical analysis.

Data was analysed using SPSS version 20, ANOVA test was used for comparing different groups. P< 0.05 was considered significant.

3. Results and Discussion

Table 1: Scavenging activity and anti-inflammatory activitiesMean ± SD (n=3).

Plant extarcts	IC_{50} values (µg/ml) scavenging activity	IC_{50} values (µg/ml) of albumin denaturation
1-Walnut leaves (*WL*)	98.14±0.01	6.83 ±0.01
2- Flaxseed (*FL*)	35.22±0.05	9.17 ±0.03
3- Chamomile (*CH*)	37.70±0.03	6.25 ±0.00
4- Sage (*SA*)	37.20±0.03	7.67 ±0.00
5- Arnica (*AR*)	39.38±0.01	8.92 ±0.01
6-Evening primrose (*ER*)	49.85±0.09	10 ± 0.00
Ascorbic acid (Control)	84.44±0.01	---------
Aspirin (100 µg/ml)	----------	142.08±0.03

Table 2: Antimicrobial activity of different extarcts against (MERSA), n=3, (Mean ± SD).

Diameter of inhibition zone (mm)					
Plant extarct	5mg	25mg	50mg/	100mg	200mg
1-Walnut leaves (WL)	Negative				
2- Flaxseed(FL)					
3- Chamomile (*CH*)					
4- Arnica (AR)					
5- Evening primrose (EP)					
Negative control (DMSO)					
6- Sage (*SG*).	Negative		10±0.00***	11±0.00***	20±0.00***
Positive control (Vancomycin 30µg/ml)	16.3±0.47	14.67±0.58***	15±0.00***	17±0.00**	20±0.00***

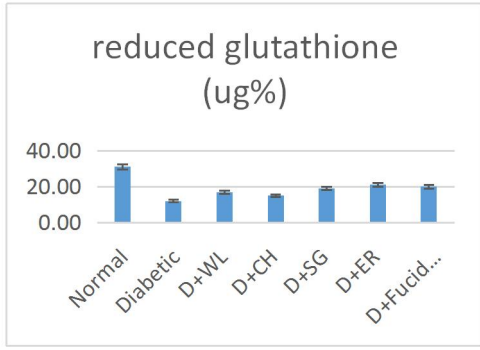

Fig 1: level of reduced glutathione in different studied groupd (Mean± SD)

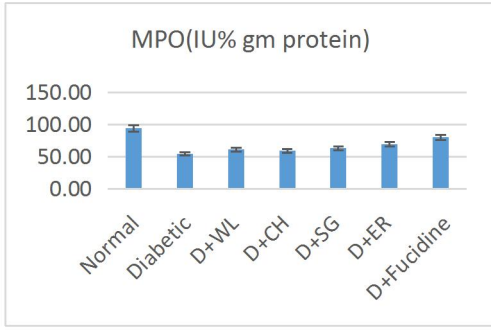

Fig 2: Activity of myleoperoxiadse (MPO) in different studied groupd (Mean± SD)

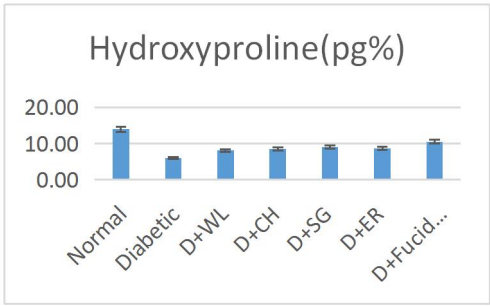

Fig 3: level of hydroxyproline in different studied groupd (Mean± SD)

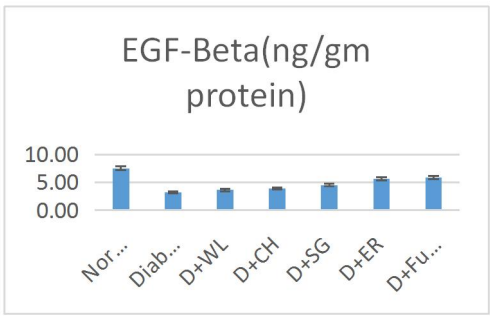

Fig 4: level of EGF-β in different studied groupd (Mean± SD)

Radical scavenging activity obtained from Saudi plants extracted with a different organic solvent was represented in Among the diverse plants extracted with a different solvent were showed significant differences ($p < 0.05$) for free radical scavenging activities in Saudi plants extracts. DPPH indicated the antioxidant potential of the extract. The IC50 values for DPPH radical scavenging of the extract of most plant exhibited variations response for radical scavenging activity (**Table 1**).There are several possible explanations for this result. WA showed highest scavenging property compared with other extracts. The bioactive compounds could enhance antioxidant activity, such as phenolic acid, phenolic alkaloids, phenolic terpenoids, phenolic glycosides, flavonoids, diterpenes tannins, curcuminoids, lignans, stilbenes and quinones [15-16]. Furthermore, the overall composition of extract achieved be different parts of the plant used, harvesting time, plant species, type of storage and drying methods [17]. Another possible explanation is that the solvent used in the extraction process, the sample preparation technique, time of extraction and temperature could affect the concentration of bioactive compounds in plant extracts, then affecting their antioxidant activity [15-17].

Therefore, the alteration of proteins is identified as a sign of inflammation [18-20]. In current study, the highest inhibitory effect of IC50 against protein denaturation was detected for ER. Anti-inflammatory activity obtained half maximal inhibitory concentration (IC50) of various plant extracts with a different solvent. These Saudi plant extracts demonstrated significantly greater protein protection compared to aspirin. Antioxidants play a crucial role in protecting the body against damage caused by free radicals' attacks on cells [21]. Data in (table 1) showed a preliminary screening of anti-inflammatory and antioxidant activities of six plants extarcts. In

table 1 it was indicated that , chamomile, walnut leaves and sage with IC_{50} values of (6.25, 6.83 and 7.67) respectively were very highly significant different as anti-inflammatory compared to aspirin as a positive control, may be due to they have many terpenoids compound. In other hand, the oils of arnica, flaxseed and evening primrose have same effect and similar values and have an anti-inflammatory property. The results demonstrated that the use of different plants affected the anti inflammatory capacity .The anti-inflammatory is possibly mediated by anti inflammatory mediator or prostaglandins [23]. In addition, the presence of flavonoids in the plant have potential inflammatory properties [24]. Phenol and flavonoids, tannins compounds play an essential function in inflammation [25].

In previous study, evening primrose oil had significant anti-inflammatory effects on arthritic rats [22]. In table 2, the flaxseed, chamomile, sage, arnica and evening primrose had significantly higher radical scavenging activity than ascorbic acid as a positive control with IC_{50} (84.44µg/ml) whereas walnut have poor activity with IC_{50} value 98.14, may be because of the low amount of phenolic.

In (table 2) which provided antibacterial activity of different essentials oil. The results showed the negative control of DMSO showed no inhibition effect on the growth of MRSA and the positive control (Vancomycin) induced inhibition zones 16.3 mm. The average of zones of inhibition of sage and evening primrose with zone 20 at 200mg/0.5ml had very high significant differences compared to positive control whereas they do not have inhibition zones at concentration 5mg/0.5ml. The oils walnut, flaxseed, chamomile and arnica at different concentration have no inhibition zones against MRSA.

Sage (*SG*) showed most antimicrobial effect among other extarcts [26]. By tradition, people have been widely using it to treat skin disorders, the regulation of menstruation, and a diuretic [27] [table 2].

Skin injury or wound is either a cut or opening of the skin, located near the surface or deeper in the skin in some diseses and accidents. Diabetes complications are the most problems of uncontrolled glycemic index. It affects about 3000 million people worldwide [28]. Frequent infection susceptibility are most clinical signs. Previous studies showed that, delayed wound healing in diabetics may be due to decrease cell proliferation, sepsis, lowered cell surviving, and decreased wound contraction [29]. The inflammatory mediators response to healing site as tranfroming growth factor (TGF-β) is also dcreased mostly in uncontrolled patients [30]. Wound healing abnormality is common complication in uncontrolled diabetics may be due to decreased collagen synthesis or growth factors [31].

Wound healing is a multipart and cascade of molecular interactions comprising three major phases: inflammatory, proliferative, and remodeling[32].The present treatment options for wound healing remain a global health challenge [33]. Saudi Arabia has the wealthiest ecological areas in the Arabian Peninsula and providing precious crops and medicinal plants . Further, It demonstrated that the Src and Syk kinases contributed to the anti-inflammatory activities, recommending it as a novel natural anti-inflammatory [32,33].

Data obtained in figures (1-4) showed that, a significant decreased in the elevels of hydroxyproline ($p<0.001$) content , reduced glutathione($p<0.01$) , MPO ($p<0.01$) and EGF-beta ($p<0.001$) in diabetic rats comapred with normal contral. Diabetic rats treated with either extarcts of Walnut leaves (*WL*), Flaxseed (*FL*), Chamomile (*CH*), Sage (*SA*), Arnica (*AR*), Evening primrose (*ER*) showed improvement in these parameters compared with untreated ($p<0.001$). But the ER is the most potent than other extract. Elevated hydroxyproline reflect collagen synthesis, that enhanced wound healing .Neutrophils also have a role of recruiting macrophages to the wound site. Myeloperoxidase (MPO) play a role in inflammatory phase of wound healing.

Sage (SG) had rich antioxidant activity compared to the other seven plant extracts, associated with effective antimicrobial activity against five test microorganisms [34]. Those results suggested that this plant's antioxidant, antimicrobial activity may contribute to their claimed wound healing.

Dual treatment with topical neem oil and oral use of haridra powder capsules were found effective for chronic non-healing wounds. PR extract could result in synergism, with improvements in wound healing in a shorter time. *Sage* extracts displayed potent antibacterial and antioxidant activities [35]. Additionally, the topical ointments were prepared from *Urtica dioica* and other plants, resulting in efficient wound healing properties [36-39].

4. Conclusion

It was concluded that, Sage (*Salvia officinalis*) showed antimicrobial against MERSA at high concentartions compared with other extarcts. The Walnut leaves demonstrated the maximum one. While Evening primrose showed potent inflammatory effect and potent wound healing in diabetic rats.

5. Conflict of interest.

The authors declare that, there is no conflict of interest.

6. Funding

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