EVALUATION OF ETHANOLIC CRUDE EXTRACTS OF AZADIRACHTAINDICA AND
SOLANUMNIGRUM EFFICACY FOR DERMAL WOUNDS HEALING

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Abstract

The herbal formulations based on ethanolic crude extracts of Azadirachta indica and Solanum nigrum revealed effectual antimicrobial activity during recovery of mice dermal wounds. For further confirmation, agar well diffusion method was used to estimate the antimicrobial efficacy of A. indica and S. nigrum against the microbial strains of Staphylococcus aureus, Escherichia coli and Candida spp. and remarkable growth inhibition zones were observed with average diameter of 11.66mm, 13mm and 10mm for A. indica whereas 8.33mm, 10mm and 13.33mm for S. nigrum, respectively. Moreover, GC-MS results of crude ethanolic extracts’ compositional analysis support that A. indica contains a significant amount of oleic acid which stimulates collagen fibers formation and wound recovery. Similarly, a considerable amount of antioxidants which were phenol and benzene based derivatives were also noted in crude ethanolic extract of A. indica whereas in S. nigrum the major constituents were octane based derivatives which exhibited the pharmacognosial role for dermal wounds recovery. These features highlight that researchers should further focus on dose optimization of these specific herbal formulations for skin wounds healing and regeneration at human level to replace xenobiotic drugs.

Keywords: Antimicrobial Efficacy, Antioxidants, Dose Optimization, Dermal Wounds, Xenobiotic Drugs.

1. Introduction

Skin is the prime component of our body and serves as a protective shield for whole internal physiological and metabolic machinery from outer milieu. As an outcome it is directly exposed to diverse types of wounds, toxic effects inducing particulate matter and broad spectrum of solar radiations. It is not only the receiver of different external stimulations for instance thermal variations and its homeostatic management; the dermal layers also prevent loss of valuable body fluids like blood and exterior intrusion of toxicants, pathogenic organisms and additional vast range of stimuli (Mecheleidt et al., 2002; Cork et al., 2006). Whereas progressive rise in pollution is directly increasing rate of dermal disorders’
occurrence. It is assured that synthetic drugs are serving well but they commonly produce the side effects. Consequently, dermatologists and herbal researcher are trying to explore natural cures other than artificial ones in the modern age.

The study of dermal injuries and induced cuts is of immense worth because it is the major constituent of the body which has a well-developed scheme of protection and on exposure of any injury, infection or soreness also provides a chief route of transmission to pathogens. That is why, animal model based researches are frequently held to scrutinize the effect of experimental selected drugs on skin regeneration and healing phases with least negative effects and offer option(s) of improved medicinal pursuits. Interruption in physical consistency of skin may cause diverse effects, from negligible to even lethal outcomes (Adam et al., 1999). Whereas the most advantageous skin restoration and revival is an intricate process of interlinked physiological and molecular phases in which occurrence of resettlement and propagation of cells and reorganization of extracellular matrix takes place. A well-organized wound management involves dose optimization of ache reducing drugs, application of anti-inflammatory, externally or orally or injected antimicrobial mediators and other medicinal stimuli for boosted dermal regeneration and wound healing (Thakur et al., 2011).

The human herbal cure for several diseases is in use since ancient times (Hammer et al., 1999). It is due to the presence of diverse types of herbal ingredients which are important bioactive antimicrobial molecules. In present age, excessive use of commercial antibiotic and synthetic chemicals either for cure of human, animals or for agricultural purposes is threatening to human health, ecosystem and environment. The natural resistant potential of therapeutic plants against pathogenic organisms works as motivational source for the researchers to find and screen their effective components and their targeted metabolic mode of actions. As an outcome, operation of remedial herbs is prevailing for treatment of pathogenic ailments by searching innovative pharmacognosal antimicrobial constituents (Bisignano et al., 1996; Lis-Balchin and Deans, 1996; Moaz and Neeman, 1998; Hammer et al., 1999; Das et al., 2010).

The evaluation of biologically effective elements of beneficial flora is dependent on their systematic assessment and screening and examination of risks regarding their intake, establishment of effective standard dose and genuine pharmacognosal description of the freshly found medication. Till now a significant proportion of plants have been employed to their outstanding antimicrobial modes of action which have been repetitively explored round the globe, for contagious ailments cure (Tanaka et al., 2006).
The function of herbal drugs in wounds and skin disorders’ healing is investigated to get for a number of important results (Shivhare et al., 2010). Such as, Solanum nigrum (European Black Nightshade) and Azadirachta indica (Indian Lilac) have been described as effective healers of skin cuts and injuries (Shtayeh et al., 1998; Shale et al., 1999; Rajendran et al., 2003). All floral parts of S. nigrum have been documented as effectual recovering elements in duration of experimental assessments involving both animal model based studies as well as from medical data of wrinkles reduction and for healing of rat bite, dermal epidemics, cuts, wounds, sprains and inflammation (Rajendran et al., 2003).

In reported data, the anthelmintic, antifungal, antibacterial, and antiviral ingredients of the A. indica were well acknowledged: nimbin, nimbinin and nimbidinasare the major potent antibacterial components (Siddiqui, 1942; Sidhu et al., 2004). Whereas the most important active constituents of S. nigrum include: Glycoalkaloids (solamargine, solasonine, solanine and solanidine) (Lin et al., 2007; Glossman-Mitnik, 2007), glycoproteins, polysaccharides, polyphenolic compounds such as gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin (Sikdar and Dutta, 2008). It has been observed that Solanum nigrum contains such hormonal steroids which boosts up course of regeneration and anti-inflammatory activity and displays antitumor mediation by raising the rate of apoptosis of tumors in mice (Zakaria et al., 2006; Li et al., 2007; Wang et al., 2007).

2. Materials and methods

2.1 Plants’ Collection and ethanolic crude extracts’ formation

Sample plants of Azadirachta indica and Solanum nigrum were collected from diverseregions of Chakwal (Punjab, Pakistan). The leaves of A. indica and S. nigrum were dried separately and after grinding, their obtained powders were stored in air tightened clean and labeled jars. Then 750 g of the each material was mixed with 4500 ml of ethanol in separate glass containers. Later on, they were let to stand for 7 days. In the next step, their filtration and then distillation under vacuum were carried out to gain concentrated ethanolic extract. The ethanolic extract of both species’ leaves were stored in desiccators for additional phytochemical and pharmacological screening (Purohit et al., 2013).

2.2 Determination of antimicrobial activity

To explore the effective antimicrobial spectra of herbal ethanolic extracts of A. indica and S. nigrum, agar well diffusion assay was used. For this purpose, the selected test
organisms were Staphylococcus aureus, Escherichia coli and Yeast (Candida sp.). Each agar well was of 6 mm diameter and 50µl of 40% each of the plant extract aqueous solution was poured in properly labelled well. Later on, they were incubated overnight at 37°C and the diameter of growth inhibition zones of each microbial strain was measured (Irshad et al., 2012).

2.3 GC-MS analysis of crude ethanolic extracts

The GC-MS analysis was performed using Aligent Gas Chromatograph 5890 linked to Aligent 5973 inert MSD, equipped with DB-35 column. The selected working principle was split injection and set flow rate of carrier Helium gas was 1ml/min. The initial temperature programming was 60°C for 3minutes, and end up to 260°C (10°C/min.) with 5minutes interval with column interference was at 280°C whereas ionization source was at 250°C. The electron ionization (EI) mode was set at 70eV to record mass spectra. The identification was performed on Wiley and NIST MS data library using MSD Chemstation software (Kanthal et al., 2014).

3. Results

3.1 Antimicrobial spectra

It was noted that A. indica has broader range of antimicrobial activity than S. nigrum (Figure 1) and statistical analysis also supported these results as A. indica was found comparatively more effective against E. coli than for S. aureus and Candida sp. (Table 1). It was observed that zones of growth inhibition were clearly broader around the agar wells of A. indica than S. nigrum. But for yeast strain, the case was found reverse (Figure 1). Whereas maximum antimicrobial activity against Candida sp. was shown by S. nigrum than for E. coli and S. aureus (Table 1). This significant difference in antimicrobial activity of S. nigrum for selected strains can also be noticed in the form of varying diameters of zones of inhibition (Table 1).
Table 1: Comparison of herbal antimicrobial activity

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Selected Strains</th>
<th>Average size of inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A. indica</td>
</tr>
<tr>
<td>1.</td>
<td>Staphylococcus aureus</td>
<td>11.66**</td>
</tr>
<tr>
<td>2.</td>
<td>Escherichia coli</td>
<td>13**</td>
</tr>
<tr>
<td>3.</td>
<td>Yeast Candida sp.</td>
<td>10</td>
</tr>
</tbody>
</table>

*Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and at 0.01% level significant difference (**) and at 0.1% level significant difference (*) were found.

3.2 GC-MS estimation

3.2.1 Constituents’ Qualitative analysis of crude ethanolic extract of A. indica

In this sample, the retention time of vaporization was lowest for cyclohexanol, 1-methyl-4-methylethenyl-acetate of 5.836 minutes whereas maximum retention time was taken by oleic acid of 19.988 minutes. The retention time of other constituents were found between these two upper and lower peak values (Figure 2).

3.2.2 Constituents’ Quantitative analysis of crude ethanolic extract of A. indica

In this regard, among 12 screened components, maximum percentage of 62.421% was noted for Benzene,1-methoxy-4-1-propenyl whereas significant amounts of Benzaldehyde,4-
methoxy 6.847% and Phenol, 4-2-propenyl-acetate 6.808% were also found in crude ethanolic extract of A. indica. Moreover, minimum amount of 0.752% for Bicyclol-3-1-1-hepto-2-ene, 2, 6-dimethyl-6-4-methyl-3-pentenyl was detected by GC-MS (Figure 2).

![Figure 2: Graphical representation of GC-MS results for ethanolic crude extract of A. indica](image)

### 3.2.3 Constituents’ Qualitative analysis of crude ethanolic extract of S. nigrum

In this sample, the retention time of vaporization was lowest for Pentadecanoic acid, 1-4-methylester of 17.066 minutes whereas maximum retention time was taken by 9, 12, 15-Octadecalrienoic acid, (2, 2, 2) - of 20.422 minutes. The retention time of other constituents were found between these two upper and lower peak values (Figure 3).

### 3.2.4 Constituents’ Quantitative analysis of crude ethanolic extract of S. nigrum

In this regard, among 6 screened components, maximum percentage of 44.538% was noted for 9,12,15-Octadecalrienoic acid,(2,2)-m ethylester whereas significant amounts of 9,12,Octadecadeionic acid, ethylester 23.598% and n-Hexadecanoic acid 21.593% were also found in crude ethanolic extract of A. indica. Moreover, minimum amount of 1.867% for 9, 12, 15-Octadecalrienoic acid, (2, 2, 2) was detected by GC-MS (Figure 3).
Figure 3: Graphical representation of GC-MS results for ethanolic crude extract of S. nigrum

Table 2: GC-MS evaluation report for A. indica and S. nigrum crude ethanolic extract

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Compounds found</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.836</td>
<td>Cyclohexanol, 1-methyl-4-(1-methylethenyl)-acetate</td>
</tr>
<tr>
<td>9.389</td>
<td>Arisole, p-allyl-</td>
</tr>
<tr>
<td>10.907</td>
<td>Benzene, 1-methoxy-4-(1-propenyl)-</td>
</tr>
<tr>
<td>11.209</td>
<td>Benzaldehyde, 4-methoxy-</td>
</tr>
<tr>
<td>11.46</td>
<td>Bicyclo(3.1.1)hept-2-ene.2.6-dimethyl-6-(4-methyl-3-pentenyl)-</td>
</tr>
<tr>
<td>12.818</td>
<td>2-Propanone, 1-(4-methoxyphenyl)-</td>
</tr>
<tr>
<td>13.507</td>
<td>Benzhydrazide, 3-methoxy-N2-(allylaminothiocarbonyl)-</td>
</tr>
<tr>
<td>13.862</td>
<td>1-Propanone, 1-(4-methoxyphenyl)-</td>
</tr>
<tr>
<td>14.311</td>
<td>2,3,4-Trimethoxycinnamic acid</td>
</tr>
<tr>
<td>15.649</td>
<td>Phenol, 4-(2-peopenyl)-acetate</td>
</tr>
<tr>
<td>17.803</td>
<td>n-Hexadecanoic acid</td>
</tr>
<tr>
<td>19.988</td>
<td>Oleic acid</td>
</tr>
<tr>
<td>17.066</td>
<td>Pentadecanoic acid, 14-methyl-, methylester</td>
</tr>
<tr>
<td>17.736</td>
<td>n-Hexadecanoic acid</td>
</tr>
<tr>
<td>19.153</td>
<td>9,12-Octadecadienoic acid(2.2)-, methylester</td>
</tr>
<tr>
<td>19.933</td>
<td>9,12-Octadecadienoic acid, ethylester</td>
</tr>
<tr>
<td>20.235</td>
<td>9,12-Octadecadienoic acid(2.2)-, methylester</td>
</tr>
<tr>
<td>20.422</td>
<td>9,12,15-Octadecadienoic acid(2.2.2)-</td>
</tr>
</tbody>
</table>
4. Discussion

Antimicrobial data elaborated the considerable growth inhibitory role of both selected herbs against the strains of S. aureus, E. coli and Yeast Candida spp. (Asif, 2012). A. indica was found more effective antibacterial agent than S. nigrum as wider zones of growth inhibition were recorded for it against S. aureus and E. coli as comparable reports have already been documented (Almas, 1999; Jain et al., 2010; Maragathavalli et al., 2012; Padal et al., 2013; Purohit et al., 2013; Pandey et al., 2014). That is why; formulations based on crude ethanolic extract of A. indica are considered as potent antimicrobial and dermal regenerating agent to cure skin sore, wounds and vast range of dermal infections (Soudahmini et al., 2005; Pandey et al., 2014; Javed, 2016; Javed and Qazi, 2016). Furthermore, to get pharmacognosal antibiotic effects of A. indica for dermal diseases, its optimized amount can be added in herbal drugs (Hafiza, 2000). Whereas S. nigrum showed better antimicrobial activity against the selected strain of Candida sp. following the agar well diffusion triplicate method. Similar findings have earlier been described (Ravi et al., 2009; Atanu et al., 2011). Moreover, Javed and Qazi (2016) have recently reported that S. nigrum possesses remarkable antimicrobial properties to heal and regenerate albino mice skin wounds.

The results of GC-MS analyses of both ethanolic crude extracts provided both qualitative and quantitative data of retention time and peak area, respectively (Joshi et al., 2011; Raphael et al., 2012). In the extract of A. indica, oleic acid and a variety of benzene and phenol derivatives were identified (Gopalakrishnan and Vadivel, 2011; Ferreira et al., 2012; Aparnaand Subha, 2014; Runde et al., 2015). Hinz (2007) reported that oleic acid enhances the collagen fibers deposition at wounded sites of skin. It also serves as stimulus for the activation of endothelial cells and initiates the expression of adhesion molecules, in short, channelizes the accumulation of phagocytes in the inflamed targeted site and triggers systematic apoptosis and necrosis to clear out all cell debris (Cassatella, 1999; Cury-Boaventura et al., 2004). Similarly, Nevin and Rajamohan (2010) reported that phenolic compounds are ideal skin wound healers. Another research report also highlighted that the application of benzene and phenol based derivatives are potent antioxidants and oxidative damaged of wounded skin tissues (Phan et al., 2001). Whereas the presence of octane derived compounds in the extract of S. nigrum was detected in higher ratio (Ravi et al., 2015) which might be the potent agent for skin wounds healing. Many pharmacognosal uses of these constituents which are present both in A. indica and S. nigrum are already well documented for their wound healing, antimicrobial and anti-inflammatory potentials (Vassiliou et al.,
2009; Jain et al., 2011; Sun et al., 2014). The prospective of these selected herbs for dermal healing and recovery is needed to be further investigated; specifically in the form of their beneficial constituents calculated fractions present in the plants which are collected from the different soils and which may be at diverse physiological stages. Therefore the screening of the discrete components and their in vitro and in vivo estimations for stimulating the course of skin restoration and wound cure will probably provide innovative approaches of injuries, cure and overall dermal health management in a natural manner.

5. Conclusion

In the light of above estimation, it can be concluded that due to the presence of antimicrobial mode of action bearing ingredients, the both selected herbs should be further analyzed and tested for pharmacological pursuits by dose optimization for the dermal wounds healing and regeneration as an suitable alternative of xenobiotic drugs which exhibit side effects.

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References


