Ilorin Journal of Science
FAPSCON 2024 PROCEEDINGS, 2024, pp. 280–288 (Printed in Nigeria)
ISSN: 2408 – 4840 © 2024 Faculty of Physical Sciences, University of Ilorin
https://doi.org/10.54908/iljs.fapscon.2024.11.03.1037

ILORIN
JOURNAL OF SCEINCE

ILJS-24-087 (SPECIAL EDITION)

Pharmacognostic Evaluation, Antimicrobial activities and Phytochemical Screening of Selected Medicinal Plants used in Folkloric Medicine for Wound Treatment

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Abstract

Plants have been used for thousands of years to treat wounds. Poly herbal formulations (PHF) are efficacious in enhancing therapeutic effectiveness and reducing toxicity of individual plants. *Ocimum canum* and *Balanites aegyptiaca* were combined in ratio of 1:3; and extracted with ethanol. Physicochemical evaluations were carried out on the individual plants and their combination. In addition, the extract of the polyherbal formulation was subjected to qualitative, quantitative phytochemical and antimicrobial analysis. The results obtained from the qualitative phytochemical screenings indicate the presence of tannins, saponin, terpenes, steroids, flavonoids and alkaloids in the extract. Quantitative analysis revealed total flavonoids, phenols, and tannins present in the combined herbal extract are 295.133mg/g, 71.8644mg/g and 29.2375mg/g respectively. The extracts of the combined plants exhibit some significant level of antimicrobial activities. The significant amount of tannins and flavonoids in the PHF extract suggests the antimicrobial properties of the combination and can be an effective remedy for wound infection and healing.

Key Words: Pharmacognostic evaluation, Phytochemical evaluation, antimicrobial activity, *Ocimum canum, Balanites aegyptiaca*

1. Introduction

Physical injuries resulting in a break or opening in the skin are called wounds. The restoration of the skin's altered functional status and anatomical continuity depends on the wound healing process (Tottoli *et al*., 2020). Inflammation, cell migration, and proliferation are the intricate processes that accompany wound healing and fibroblast cells play a very important role in all these processes (Addis *et al*., 2020). Though healing process occurs naturally, an infection, mostly from *Staphylococcus aureus*, *E. coli*, *Psuedomonas* spp. and *Bacillus* spp. can seriously delay it by prolonging the inflammatory phase, disrupting the normal clotting mechanisms and ultimately delaying angiogenesis (Cavallo *et al*., 2024). Various medicinal plants used in folk medicine have demonstrated wound healing property, and some of these species are currently used in commercial preparations (Frankova *et al*., 2021; Gould *et al*., 2015).

Balanites aegyptiaca (L.) Delile also known as desert date is native to dry land areas of Africa and South Asia. Almost all the parts of the plant are traditionally used in several folk medicines acting as a fumigant to treat circumcision wounds (Shahzard A and Sahai A, 2013). It has potent wound-healing

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activity. The use of the kernel oil for the treatment of wound has been reported from Nigeria (Breyer J.M and Brandwijk M.G, 1982). In Libya and Eritrea, the leaves are used for cleaning infected wounds. Pharmacological properties of the plant include; antioxidant, antibacterial (Kahsay *et al*., 2014), antidiabetic, anti-inflammatory, antiviral, analgesic and wound healing activity (Chothani and Vaghasiya, 2011). The paste of shoot is utilized for dressing of wound. The seed oil is used to treat tumors and wounds (Khalid *et al*., 2007).

The genus Ocimum, belonging to the Lamiaceae family, contains approximately 50-150 species and is found in tropical and subtropical regions of Asia, Africa, and Central and South America (Runyoro *et al*., 2010). The essential oils obtained by hydrodistillation or steam distillation from these aromatic and medicinal herbs were shown to possess good antimicrobial activities against a wide range of microorganisms (Carovic-Stanko *et al*.,2010: Runyoro *et al*., 2010) and also antioxidant activities (Dambolena *et al*.,2010: Kwee and Niemeyer., 2011).

Historically, almost all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. Medicinal plants formed the basis for either total development or templates upon which modern western medicines are based (Kayser, 2018). Majority of these involve the isolation of the active constituents found in a particular medicinal plant and its subsequent modification. In the developed countries of the world, 25 percent of the pharmaceuticals are based on plants and their derivatives; this use of medicinal plants is well known and tolerated among the indigenous people in rural areas of many developing countries (Barbhuiya *et al*., 2022). In this region, sophisticated care is often unaffordable, where many patients rely on familiar, readily accessible and in expensive traditional wound healing remedies (Gould *et al*., 2015). Traditional medicines, known for their effectiveness, safety, and affordability, are increasingly popular among people in both rural and urban communities. Numerous cases have been reported where traditional treatments have succeeded, especially in instances where conventional medications have not been effective.

Cases of chronic sores which would have led to amputations (Kayani *et al*., 2015; Soukand and Pieroni, 2016) have been successfully managed with traditional medicines. Several studies have documented the wound healing potentials of herbs from different parts of the world (Villegas *et al*., 1997; Gomez-Beloz*et al*., 2003; Kumar *et al*,. 2007; Dande and Khan, 2012; Saroja *et al*., 2012; Tepole, 2017; Biswas *et al*., 2017; Na *et al*., 2017). Nevertheless, chronic wounds in southwestern Nigeria rarely led to amputation because of the rich indigenous experience in handling such with herbal medicines. The concept of polyherbalism in which products with combined extracts of plants are considered more effective rather than individual ones (Phougat *et al*., 2022). Polyherbalism results in cheaper medications by reducing the duration of therapy or individual cost for antimicrobial medications.

The present study was undertaken to pharmacognostically evaluate, investigate the phytochemical screening and explore the antimicrobial activity of polyherbal composition from *Ocimum canum* and *Belanites aegyptiaca* in the ratio of 1:3 based on ethnomedicinal survey reported as used in wound healing.

2. Materials and Method

Plant collection and extraction

The dried leaves of *Ocimum canum* and dried stem bark of *Belanites aegyptiaca* were collected and identified by Muazzam Ibrahim, a staff of the Department of Medicinal Plant Research and Traditional Medicine at the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja.

The plants were individually extracted then in combination by maceration method using ethanol. The choice of the solvent was influenced by the ethnomedicinal survey report.

2.1 Physicochemical charac*terization*

Physicochemical parameters such as moisture content, total ash, alcohol soluble extractive value and water-soluble extractive value were determined following African Pharmacopoeia (1986) and WHO standard on the plants singly and in combination.

2.2 Phytochemical analysis

Phytochemical screening of the Polyherbal formulation extract was carried out to determine the presence of carbohydrate, alkaloid, tannins, saponins, terpenes, steroid, glycosides and flavonoid using standard methods described by Adigwe *et al*., 2021

2.3 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum concentration of extracts of the polyherbal formulation was carried out by micro-broth dilution method according to method described in NIPRD SOP.
The Minimum inhibitory concentration (MIC) of extracts was carried out by micro-broth dilution method. The 96-microtiter well was prepared by dispensing 50 µL of tryptic soy broth from row 2-12. One hundred (100 µL) from the stock solution of extract was added into the first well followed by two-fold serial dilution down the remaining wells. The wells (1-12) were inoculated with 50 µL of standardized culture of organisms. The last row of wells did not contain the extract thus serving as organism viability control (OVC). Each plate was shaken for 20 seconds (with a shaker at low speed) and incubated at 37^{o}C for 24 hrs. After incubation period, the wells were added 25 µL of tetrazolium dye, the plates reincubated for 2 hrs and observed for absence or presence of microbial growth by colour change in the wells. The MIC was defined as the lowest extract concentration that prevented the color change of the tetrazolium dye to purple. Colourless well was interpreted as no bacterial growth and purple color was interpreted as growth occurrence. The test was performed in triplicate.

2.4 Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by pipetting 50 µL from the wells used to determine the MIC and sub cultured onto freshly prepared Mueller hinton agar. The plates were incubated at 37^{o}C for 24 hrs the least concentration at which the organism did not grow was taken as the MBC.

3. Result and Discussion

Table 1: Results of Physicochemical evaluations of *Ocimum canum* leaves, *Balanites aegyptiaca* and PHF

S/N	PARAMETERS	PERCENTAGE MEAN ± SD (O.C.)	PERCENTAGE MEAN ±SD (B.A.)	PERCENTAGE MEAN ± SD (PHF)
1	Water soluble extractive value	23.33 ± 7.50	7.66 ± 0.57	12.00 ± 0.00
2	Alcohol soluble extractive value	13.33 ± 1.52	9.00 ± 0.00	19.00 ± 7.81

3	Total Ash value	9.66 ± 0.76	8.00 ± 1.00	9.16 ± 0.57
4	Moisture content	11.66 ± 0.14	8.08 ± 0.14	8.52 ± 0.00

Key note: O.C.= *Ocimum canum* B.A. = *Balanites aegyptiaca* PHF = Polyherbal formulation

Table 2: Qualitative Phytochemical Screenings of PHF

Phytochemicals	Results
Carbohydrates	-
Tannins	+
Glycosides	-
Saponin	+
Terpenes	+
Flavonoid	+
Alkaloid	+
Steroid	+

Key Note: + = Present - = Absent

Table 3: Quantitative Phytochemicals Screening of PHF

Quantitative analysis	Unit Expressed	Amount ± SD
Total Flavonoid content	Mg QE/g	295.133 ± 0.6599
Total Phenolic content	Mg GAE/g	71.8644 ± 16.17959
Total Tannin content	Mg/g	29.2375 ± 13.78236

Table 4: Antimicrobial activity of Polyherbal formulation (PHF) extract Minimum Inhibitory Concentration (MIC)

Bacterial isolates	PHF	Control (Chloramphenicol)
S. aureus	NA	0.39
E. coli	12.5	1.56
S. paratyphi	12.5	0.20
K. pneumonia	3.13	0.39
P. aeruginosa	25	1.56
C. albicans	12.5	NA

Keynote NA= no activity

Table 5: Minimum Bactericidal Concentration Values (MBC) (mg/mL)

Bacterial isolates	PHF
S. aureus	NA
E. coli	25
S. paratyphi	25
K. pneumonia	6.25
P. aeruginosa	50
C. albicans	25

Keynote NA= no activity

4. Discussion

The result of the phytochemical screening reveals the presence of Tannins, Saponins, Flavonoids, alkaloids, steroids and Terpenes. Carbohydrates and glycosides were absent. The phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of medicinal plants (Edeoga *et al*., 2005). The leaves of *ocimum canum* are rich in flavonoids, saponins and tannins, with considerable amount of phenolics and alkaloids (Aluko *et al*.,2012). Flavonoids are polyphenolic compounds that are biologically active against liver toxins, microorganisms, inflammation, tumor and free radicals (Okwu, 2004). Saponins are natural glycosides that act as hypoglycemic, antifungal and serum cholesterol lowering agents in animals (Sapna *et al*., 2009), Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess anti-diuretic and antidiarrhea properties (Okwu, 2004). Phenolic compounds are potent antioxidants and free radical scavengers with inhibitory activities against some pathogenic microorganisms (Khoobchandani *et al*., 2010). Dorothée *et al* in 2019 also reveal the presence of flavonoids and terpenoids in *O. canum*. Research conducted by Hokasatte *et al* in 2020 report the presence of flavonoids, saponins, alkaloid and steroid in the stem back of *Balanites aegyptiaca* (Hokasatte *et al*., 2020). Also, preliminary phytochemical screening of the work done by kulawe in 2019 revealed the presence of alkaloids, flavonoids, tannins, saponins and steroids in the roots and stem bark, while anthraquinones were present in the leaf only. (kulawe *et al*., 2019), this was also in relation to work done by Adebayo and Ishola in 2009 who reported the presence of this phytochemicals. (Adebayo and Ishola., 2009). Thus, these suggest the presence of these phytochemical constituents in the polyherbal formulation which are responsible for its wound healing ability.

It is important to set standards for all the parameters associated with pharmacognostic and physicochemical characters as these play key roles in determining the identity, purity and quality of a crude drug. These standards must be established for every crude drug to be included in herbal pharmacopoeia.

A detailed examination of pharmacognostic and proximate properties is necessary to determine the quality, identity, and purity of crude medicines. There are pharmacognostic conditions that must be fulfilled by each crude treatment to be listed in an herbal pharmacopoeia. Moisture content is one of the most important and frequently used parameters in the preparation, preservation, and storage of medicinal plants (African Pharmacopoeia, 1986). The examination of physicochemical parameters such as moisture content and ash value is necessary to establish the physiological and non-physiological properties, determine the likelihood of microbial growth or contamination, and identify the presence of contaminants (Pandey *et al*., 2015). When compared to the limit for water content (8 to 14%) for vegetable medications (African Pharmacopoeia, 1986). Each plant's moisture content profiled falls within the permitted range. Ash values and extractive values not only aid in determining the purity of crude pharmaceuticals but are reliable methods of identifying adulteration. Ash is the entire residue that is left over from medicinal plants after all moisture has been removed and all organic matter (fat, protein, carbohydrates, vitamins, and organic acids) has been burned at a temperature of about 600°C. The total ash values of the various plants that were profiled showed that they contain a significant amount of phosphates, carbonates, or both.

A higher extractive value was obtained for the aqueous solvent of *Ocimum canum* (23.33%) than alcohol (13.33%) suggesting the possibility of the presence of more polar constituents (Tab. 1). The moisture content observed for *O. canum* 11.66% is within the limit of (8-14%) set by the WHO thus indicating that the plant crude drug has a good shelf life. The total ash for *O.canum* value of 9.66% is indicative of low inorganic contents though the values are subject to factors such as the soil type, mining and construction activities around the area of cultivation. A higher alcoholic value of (9%) than aqueous 7.66%) for *Belanites aegyptiaca* (B.A) suggests the possibility of the presence of more non polar constituents (Tab 1). The moisture content of 8.08% indicates a high shelf life of the crude drug and the total ash value of 8% is also indicative of the inorganic content present in the crude plant drug. For the polyherbal (PHF) formulation (PHF) of the combination of *Ocimum canum* and *Belanites aegyptiaca* , the results of the physicochemical parameter showed a higher alcoholic extractive value of (19%) than aqueous (12%). The moisture content and Total ash value were 8.52% and 9.16% which are within the limit of WHO.

Based on the MIC result, the extract tested exhibited varying degrees of inhibitory activities ranging from 25 mg/ml – 1.56 mg/ml. The PHF extracts tested at 200 mg/ml concentration inhibited the growth of *E. coli*, *S. paratyphi*, *K. pneumoniae, P. aeruginosa and C. albicans* with MICs of 12.5 mg/ml, 12.5 mg/ml, 3.13 mg/ml, 25 mg/ml and 12.5 mg/ml respectively. The PHF extract was not active against *S. aureus*, it could be possible that, the components that could inhibit the growth of these test bacteria have been destroyed during the extraction process or the concentrations employed are too low to inhibit the growth while for MBC, the extract tested exhibited varying degrees of bactericidal activities ranging from 50 mg/ml – 3.13 mg/ml. The PHF had bactericidal activities against *E. coli*, *S. paratyphi*, *K. pneumoniae, P. aeruginosa and C. albicans* with MBCs of 25 mg/ml, 25 mg/ml, 6.25 mg/ml, 50 mg/ml and 25 mg/ml respectively. Doughari *et al*., (2007) reported high significant effect of antibacterial activity at higher concentration of the extract showed appreciable inhibition. It also agrees with the work of Khalid *et al*., (2010) whose findings reported the high protective activity of ethanol and ethyl acetate crude extract of *Balanites aegyptiaca* against the test bacteria at higher concentrations. Kashari in 2021 also report appreciable activities of the stem back against Staphylococcus aureus and Shigella dysentariae.

4. Conclusion

The phytochemical screening of the PHF confirmed the presence of important phytochemicals that have been documented in literature with anti-inflammatory, anti-oxidant and wound healing bioactivities, which are likely responsible for the therapeutic effects observed in the studied plants. Pharmacognostic analysis revealed that the moisture content, ash values, and extractive values of the plants fell within acceptable ranges, indicating good quality and shelf life. Furthermore, the PHF demonstrated significant inhibitory and bactericidal activities against various pathogens, supporting its potential as an effective antimicrobial agent for wound treatment.

Recommendation

Comparative trials between the polyherbal formulation and standard wound treatment options (e.g., silver sulfadiazine) over an extended period to establish efficacy and safety profiles is recommended. Likewise, stability testing of the polyherbal formulation is recommended to ensure its efficacy over long periods, which is critical for commercial or clinical use. The investigation of the formulation's potential as an anti-inflammatory or analgesic agent in other conditions like arthritis, inflammatory bowel disease, or muscular pain will also provide an avenue for the expansion of its therapeutic applications.

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