ORIGINAL ARTICLE

Determination of biochemical constituents of *Argan* essential oil and its antimicrobial efficacy against tinea infections of *Trichophyton rubrum*

Muazzam Sheriff Maqbul[1], Abdul Rahman Ikbal[2], Tasneem Mohammed[2], Areej Dawoud[2], Aejaz Abdullatif Khan[2], S. M. Shakeel Iqubal[2], Kayamkani Abedulla Khan[3], Uday M. Muddapur[4], Gouse Basha Sheik[5], S. K. Singh[6]

[1]Faculty of Microbiology and Immunology, Ibn Sina National College of Medical Sciences, Al Mahjar Street 31906, Jeddah 21418, Kingdom of Saudi Arabia, [2]Department of General Science, Ibn Sina National College of Medical Sciences, Al Mahjar Street 31906, Jeddah 21418, Kingdom of Saudi Arabia, [3]Department of Clinical Pharmacy and Pharmacology, Ibn Sina National College for Medical Studies, Jeddah, Kingdom of Saudi Arabia, [4]Department of Biotechnology, KLE Technological University, Hubballi, Karnataka, India, [5]Deaprtment of Microbiology, College of Applied Medical Sciences, Ad-Dawadmi, Shaqra University, Saudi Arabia, [6]Department of Chemistry, GGV Central University Bilaspur, Chhattisgarh, India

Abstract

Background: The aim of this study to focus on these tinea infections caused by the mold *Trichophyton rubrum* by collecting the clinical specimens by means of skin scraping samples from the affected sites of the patients and to evaluate the susceptibility of the isolates of the obtained clinical specimens toward the *Argan* essential oil. A rapid phytochemical analysis study was also performed. **Materials and Methods:** *Argan* essential oil procured from Jeddah local market, clinical skin scrape samples from the patient. The phytochemical study was done to find out the chemical compounds present in the *Argan* essential oil, which plays a key role in determining the antimicrobial efficacy of the *Argan* essential oil. **Results and Discussion:** The interpretation of the observation and results for the *Argan* essential oil showed the promising study results. Regarding its efficacy as potential antifungal agents when compared to that of the standard synthetic chemical agents used against the clinical skin scrape isolates of *T. rubrum*. **Conclusion:** The phytochemical compounds present in the *Argan* essential oil acts as an effective remedy toward the clinical skin scrape isolates of *T. rubrum* compared to the standard antifungal agents.

Key words: Antibiotics, antifungal, antimicrobial activity, *Argan* essential oil, minimum fungicidal concentration, minimum inhibitory concentration, phytochemical properties, *Trichophyton rubrum*

INTRODUCTION

The application of the natural essential oil as an effective antimicrobial agent due to its rich phytochemical properties was in practice since the ancient times for the treatment of various infectious bacterial, fungal, and viral pathogens.[1] *Argan* essential oil is one such oil abundant in phytochemical components, especially the presence of phenolic compound, which promotes the antimicrobial activity. The *Argan* essential oil is a native of Morocco, and it is used for culinary purposes besides its medicinal applications.[2,3] The *Argan* essential oil is extracted from the fruits and nuts of a calcareous semi-desert flowering tree known as *Argan* from the botanical species of *Argania spinosa* belonging to the *Sapotaceae* family of the plant kingdom. Besides, the phenolic contents such as the catechin, epicatechin, vanillic acid, caffeic acid,

Address for correspondence:
S. M. Shakeel Iqubal, Department of General Science, Ibn Sina National College of Medical Sciences, Al Mahjar Street 31906, Jeddah 21418, Kingdom of Saudi Arabia. E-mail: shakeeliqubal@gmail.com

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tyrosol, oleuropein, and resorcinol, the *Argan* essential oil also possess a versatile source of phytochemical compounds such as carotenes, squalene, tocopherols, and a rich source of unsaturated fatty acids.[2-4] These phytochemical compounds serves as potential antimicrobial substances in combating infectious diseases.[5] The *Argan* essential oil is predominantly used especially for the treatment of superficial bacterial and fungal skin infections since ancient times with effectiveness and also used in the cosmetic industries due to its rich content of Vitamin E.[6-8] The superficial skin infections are termed as dermatophyte infections. The superficial dermatophyte bacterial infections are often caused predominantly by both the Gram-positive cocci, *Staphylococcus* and *Streptococcus* species, whereas the fungal infections are caused predominantly by the yeast *Candida albicans* or by the mold *Trichophyton rubrum*. The superficial fungal dermatophytic skin infections are referred to as tinea infections.[6-8] The tinea infections are classified based on the site of the infections such as the most common fungal infection of the feet where the initial infection can be dry and scaly followed by the secondary bacterial infection with an accumulation of soggy debris which is clinically termed as the tinea pedis commonly called as the athlete's foot.[9,10] The most common fungal infection among children is found on the scalp resulting in the baldness due to infected hair, and this condition is clinically termed as tinea capitis. The most common infections among the men were associated with beard infection, in which bacterial folliculitis is accompanied with the secondary to ingrown hairs, and this condition is clinically termed as tinea barbae.[9,10] The skin infections other than the bearded area, scalp, groin, hands, or feet tend to present as the irregular expanding rings with a raised border, and this condition is clinically termed as tinea corporis, which is commonly known as the ringworm infections of the skin. The infections of the groin, perineum, and perianal areas, which are associated with the lesions, maybe on the inner thighs, pubic, inguinal region, or scrotum were clinically termed as the tinea cruris commonly known as the jock itch. The asymptomatic skin infections characterized by the dryness with increased skin markings on the hand or the foot is clinically termed as tinea manuum.[9,10] Tinea pedis is commonly known as moccasin-type skin infections. Finally, the fungal infections of the nails known as onychomycosis, where the infections are caused by spreading of the tinea pedis infections and this condition is clinically termed as tinea unguium. The aim of this study to focus on these tinea infections caused by the mold *T. rubrum* by collecting the clinical specimens by means of skin scraping samples from the affected sites of the patients and to evaluate the susceptibility of the isolates of the obtained clinical specimens toward the *Argan* essential oil.[9,10] A rapid phytochemical analysis study was also performed to find out the chemical compounds present in the *Argan* essential oil, which plays a key role in determining the antimicrobial efficacy of the *Argan* essential oil.[11,12]

MATERIALS AND METHODS

Materials

Argan essential oil procured from Jeddah local market, clinical skin scrape samples from the patient, Saboraud's dextrose agar, peptone, KOH, and lactophenol. All the chemicals used during this research was of analytical grade. Standard antibiotics and standard HiMedia culture plates were used.

Methodology

Phytochemical analysis of the Argan essential oil

The phytochemical properties analysis of the *Argan essential oil* procured from the local market was determined by the following methodologies.[11-14]

1. Wagner's test
   A drop of *Argan* essential oil was mixed with a drop of potassium iodide and iodine resulting in the formation of a reddish brown precipitate which indicates the presence of alkaloids in the oil.
2. Ammonia and sulfuric acid mixture test
   The *Argan* essential oil was treated with dilute ammonia and concentrated sulfuric acid resulting in the formation of a yellow color, which indicates the presence of flavonoids.
3. Chloroform and sulfuric acid with acetic acid mixture test
   The *Argan* essential oil was treated with a mixture of 2 ml of chloroform and concentrated sulfuric acid with 2 ml of acetic acid resulting in the formation of a green color, which indicates the presence of steroids.
4. Xanthoproteic test
   The *Argan* essential oils were treated with a few drops of concentrated nitric acid resulting in the formation of a yellow color, which indicates the presence of proteins.
5. Ferric chloride test
   Argan essential oils were boiled with 10 ml of water in test tubes to which a few drops of ferric chloride was added to the 10 ml of heated *Argan* essential oil in a test tube to observe a blackish-blue color which indicates the presence of phenol.
6. Keller-Killani test
   The mixture of 2 ml of glacial acetic acid containing 1–2 drops of ferric chloride solution was treated with the *Argan* essential oil in a test tube, and few drops of concentrated sulfuric acid were added by placing the test tube on ice to observe the formation of a brown colored ring at the interface color indicating the presence of glycosides.
7. Benedict's test
   In a test tube, 2 ml of Benedict's reagent was treated with the *Argan* essential oil and heated gently heated to observe the formation of an orange-red precipitate indicates the presence of reducing sugar.

8. Iodine test
   The presence of iodine in the *Argan* essential oil was determined by the addition of 2 ml of iodine solution to the *Argan* essential oil which results in the positive purple colored test.
9. Ammonia test
   Dilute ammonia and conc. sulfuric acid-treated with aqueous *Argan* essential oil in a test tube to observe yellow color formation, indicating the presence of amino acids.
10. Chloroform and sulfuric acid mixture test
    The *Argan* essential oil was treated with 2 ml of chloroform and concentrated sulfuric acid resulting in the formation of a brownish-red layer, which indicates the presence of terpenoids.

Isolation and Purification of *T. rubrum*

The clinical skin scrape sample from the patients was collected by employing the aseptic scraping technique and was inoculated on a sterile Saboraud's Dextrose agar plate and incubated at 22°C for 24–48 h to observe the fungal mold colonies.

The KOH and lactophenol test was performed to observe the mold hyphae under a microscope by employing the wet mount technique as the confirmatory test.[15-17]

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test for the isolated clinical skin scrape specimens of *T. rubrum* was evaluated for the efficacy of the standard synthetic chemical antifungal agents by performing the latest rapid e-test methodology where the clinical skin scrape isolates were inoculated on Saboraud's dextrose agar plates separately, and e-test plastic strips for the respective antibiotics were impregnated and incubated at 22°C overnight to visualize the zone and ellipse, and the results were tabulated by interpreting the observed results for the interaction of the ellipse as the minimum inhibitory concentration (MIC), whereas the zone as the susceptibility of the antibiotic toward the mold.[15-18] The traditional standard antibiotic assay methods such as Kirby–Bauer disk diffusion method was employed to observe the susceptibility of the clinical skin scrape isolates of *T. rubrum* the standard disk prepared from the *Argan* essential oil extract where the mold isolates were inoculated separately on the potato dextrose agar plates along with the impregnated disks for 24 h at 22°C to observe the zone formation determining the sensitivity of the mold toward the disk. The results were tabulated and interpreted. The MIC values along with minimum fungicidal concentration (MFC) values for the efficacy antimicrobial activity of the *Argan* essential oil toward the mold were estimated by performing the standard tube dilution method where the clinical skin scrape isolates were inoculated separately in the different sets of dilutions of the essential oil in the peptone water and incubated for 24 h at 22°C to observe the no turbidity determining the sensitivity of the mold toward the acid.[19] The last dilution with turbidity determines the MIC value of the acid toward the mold. The results were tabulated and interpreted. The MFC was determined by inoculating each dilution of MIC dilutions onto the separate agar plates for each clinical skin scrape isolates of *T. rubrum* for the MIC dilutions separately. The inoculated plates were incubated for 24 h at 22°C to observe the no growth determining the sensitivity of the mold toward the *Argan* essential oil. The first dilution with no growth determines the MFC of the essential oil toward the fungal mold.[19,20] The results were tabulated interpreted.

RESULTS AND DISCUSSION

Discussion

The phytochemical analysis study was been done for the procured *Argan* essential oil to understand the reason behind its antimicrobial efficacy. The phytochemical analytical tests conducted for the *Argan* essential oil were Wagner's test, ammonia and sulfuric acid, mixture test, ammonia and sulfuric acid mixture test, xanthoproteic test, ferric chloride test, Keller-Killani test, Benedict's test, iodine test, ammonia test, and chloroform and sulfuric acid mixture test. The obtained interpretation from the phytochemical analytical test results showed the presence of chemical compounds such as alkaloids, flavonoids, steroids, proteins, phenols, glycosides, reducing sugar, iodine, amino acids, and terpenoids, respectively.[3,12,13,15] The phytochemical test results were tabulated for the reference [Table 1]. The presence of the vital phytochemical component in the *Argan*

Table 1: Phytochemical analysis of the *Argan* essential oil

Test	Observation	Compounds present
Wagner's test	Reddish brown ppt	Alkaloids
Ammonia and sulfuric acid mixture test	Yellow color	Flavonoids
Chloroform and sulfuric acid with acetic acid mixture test	Green color	Steroids
Xanthoproteic test	Yellow color	Proteins
Ferric chloride test	Violet color	Phenol
Keller-Killani test	Brown colored ring	Glycosides
Benedict's test	Orange-red ppt	Reducing sugar
Iodine test	Purple color	Iodine
Ammonia test	Yellow color	Amino acids
Chloroform and sulfuric acid mixture test	Brownish-red layer	Terpenoids

essential oil is the phenolic compounds which serves as a potential antimicrobial activity and shown the promising results against the clinical skin scrape isolates of *T. rubrum* when demonstrated with the standard antimicrobial assay techniques. A comparative analysis study was also performed for the antimicrobial activities of *Argan* essential oil extract with that of the standard antifungal agents against clinical skin scrape isolates of *T. rubrum*. The antimicrobial assay results for the *Argan* essential oil procured from the local market shown significant antimicrobial activity results against all the clinical skin scrape isolates of *T. rubrum* with an average disk diffusion of 23.05 mm zone of inhibition diameter determining the susceptibility obtained from performing the Kirby–Bauer technique with an average MIC value of 1.86 µ/ml and an average MFC value of 2.11 µ/ml. The best susceptibility for the clinical skin scrape isolates of *T .rubrum* toward the *Argan* essential oil was observed from the beard skin scrape isolates of *T. rubrum* sample with a zone diffusion of 27 mm with MIC of 1.5 µ/ml and MFC of 1 .75 µ/ml, whereas the least susceptibility was observed from the perineum kin scrape isolates of *T. rubrum* sample with a zone diffusion of 18.5 mm with MIC of 2.25.5 µ/ml and MFC of 2.5 µ/ml, respectively. The susceptibility with MIC and MFC results of the other skin scrape isolates of *T. rubrum* sample toward the *Argan* essential oil was also shown satisfactory results when compared with that of the standard antifungal agents against clinical skin scrape isolates of *T. rubrum*.[9,10] The results of the other skin scrape isolates of *T. rubrum* toward the *Argan* essential oil obtained were as follows. Feet scrape sample shown susceptibility with a zone diameter of 23 mm in disk diffusion method with MIC of 1.25 µ/ml and MFC of 1.5 µ/ml, whereas the scalp scrape sample has shown susceptibility toward with a zone diameter of 22 mm in disk diffusion method with MIC of 1.5 µ/ml and MBC of 1.75 µ/ml where the groin scrape sample shown susceptibility with zone diameter of 20 mm in disk diffusion method with MIC of 1.75 µ/ml and MFC of 2 µ/ml and the hand, nail, palm, and finger scrape samples showed the susceptibility of a zone diameter ranging from 22.5 mm to 26 mm in disk diffusion method with MIC of 1.75 µ/ml to 2.5 µ/ml and MFC of 2 µ/ml to 2.75 µ/ml, respectively**.** The presence of phenolic and other miscellaneous constituents in the *Argan* essential oil extract contributes to its rich antimicrobial content and has shown promising results in this study as well. The efficacy of the *Argan* essential oil extract against clinical skin scrape isolates of *T. rubrum* has shown excellent results when compared with that of the standard antifungal agents used in therapy. The average value of the zone of inhibition susceptibility value of the *Argan* essential oil extract against clinical skin scrape isolates of *T. rubrum* was 23.05 mm for all the skin scrape samples compared to the standard antifungal agents values of 18.88 mm for Griseofulvin, 12.55 mm for Itraconazole, 13.22 mm for Terbinafine, 20.44 mm for Fluconazole, 14.77 mm for Naftifine, 17.22 mm for Miconazole, 14.44 mm for Ketoconazole, and 12.88 mm for clotrimazole, respectively, for all the skin scrape samples assayed. The average MIC value of the *Argan* essential oil extract against clinical skin scrape isolates of *T. rubrum* was 1.86 µ/ml for all the

Table 2: Comparative chart of antimicrobial sensitive activities standard antifungal agents against clinical skin scrape isolates of *Trichophyton rubrum* by e-test study

Standard Antifungal agents	Specimens									Average Zone value
	Feet	Scalp	Beard	Groin	Hand	Nail	Perineum	Palm	Finger	
Griseofulvin	21 mm S	2 mm R	20 mm S	22 mm S	20 mm S	24 mm S	18 mm S	21 mm S	22 mm S	18.88 mm
Itraconazole	2 mm R	3 mm R	21 mm S	22 mm S	20 mm S	3 mm R	10 mm I	10 mm I	22 mm S	12.55 mm
Terbinafine	20 mm S	20 mm S	10 mm I	10 mm I	21 mm S	2 mm R	22 mm S	4 mm R	10 mm I	13.22 mm
Fluconazole	22 mm S	23 mm S	22 mm S	23 mm S	20 mm S	21 mm S	14 mm I	13 mm I	25 mm S	20.44 mm
Naftifine	9 mm I	20 mm S	20 mm S	10 mm I	10 mm I	11 mm I	23 mm S	10 mm I	20 mm S	14.77 mm
Miconazole	11 mm I	21 mm S	10 mm I	21 mm S	21 mm S	20 mm S	20 mm S	10 mm I	21 mm S	17.22 mm
Ketoconazole	4 mm R	2 mm R	11 mm I	21 mm S	10 mm I	21 mm S	21 mm S	20 mm S	20 mm S	14.44 mm
Clotrimazole	4 mm R	2 mm R	10 mm I	21 mm S	20 mm S	20 mm S	10 mm I	9 mm I	20 mm S	12.88 mm
Total Sensitive	3	4	4	6	6	5	5	2	7	
Total intermediates	2	0	4	2	2	1	3	5	1	
Total resistance	3	4	0	0	0	2	0	1	0	

Table 3: Comparative MIC values chart of antimicrobial activities of standard antifungal agents against clinical skin scrape isolates of *Trichophyton rubrum* by e-test study

Standard antifungal agents	Specimens								Average MIC values
	Feet	Scalp	Beard	Groin	Hand	Nail	Perineum	Palm	Finger	
Griseofulvin	1.25 µ/ml	1 µ/ml	0.75 µ/ml	1.25 µ/ml	0.75 (µ/ml)	1.5 µ/ml	1.25 µ/ml	1(µ/ml)	0.75 (µ/ml)	1.05 (µ/ml)
Itraconazole	1.5 µ/ml	1.25 µ/ml	1 µ/ml	1.25 µ/ml	0.75 (µ/ml)	1.5 µ/ml	1.5 µ/ml	1 (µ/ml)	0.75 (µ/ml)	1.08 (µ/ml)
Terbinafine	1.5 µ/ml	1.25 µ/ml	1.25 µ/ml	1.25 µ/ml	1 µ/ml	1.75 µ/ml	1.5 µ/ml	1.25(µ/ml)	1 (µ/ml)	1.30 (µ/ml)
Fluconazole	1.5 µ/ml	1.5 µ/ml	1.25 µ/ml	1.5 µ/ml	1.25 µ/ml	1.75 µ/ml	1.5 µ/ml	1.25 (µ/ml)	1 (µ/ml)	1.38 (µ/ml)
Naftifine	1.75 µ/ml	1.75 µ/ml	1.25 µ/ml	1.5 µ/ml	1.25 µ/ml	2 µ/ml	2.25 µ/ml	1.5 (µ/ml)	1.5 (µ/ml)	1.63 (µ/ml)
Miconazole	2.25 µ/ml	2. µ/ml	2.25 µ/ml	1.75 µ/ml	1.5 µ/ml	2µ/ml	2.25 µ/ml	1.75 (µ/ml)	1.75 (µ/ml)	1.94 (µ/ml)
Ketoconazole	2.5 µ/ml	2.25 µ/ml	2.25 µ/ml	2 µ/ml	1.75 µ/ml	2.0 µ/ml	2.25 µ/ml	2.0 (µ/ml)	2.0 (µ/ml)	2.11 (µ/ml)
Clotrimazole	2.75 µ/ml	2.5 µ/ml	2.5 µ/ml	2.25 µ/ml	2 µ/ml	2.25 µ/ml	2.5 µ/ml	2.25 (µ/ml)	2.25 (µ/ml)	2.36 (µ/ml)

MIC: Minimum inhibitory concentration

skin scrape samples compared to the standard antifungal agents values of 1.05 (µ/ml) for Griseofulvin, 1.08 (µ/ml) for Itraconazole, 1.30 (µ/ml) for Terbinafine, 1.38 (µ/ml) for fluconazole, 1.63(µ/ml) for naftifine, 1.94 (µ/ml) for miconazole, 2.11 (µ/ml) for ketoconazole, and 2.36 (µ/ml) for Clotrimazole, respectively, for all the clinical skin scrape isolates of *T. rubrum* samples assayed. The best susceptibility values recorded in the standard antifungal agents against the clinical skin scrape isolates of *T. rubrum* was with fluconazole showing the average zone of inhibition diameter of 20.44 mm with the average MIC value of 1.38 (µ/ml), but the antimicrobial assay results for the *Argan* essential oil showed better values with an average disk diffusion of 23.05mm zone of inhibition diameter with average MIC value of 1.86 µ/ml and an average MFC value of 2..11 µ/ml. Although the MIC value of the *Argan* essential oil was a little higher than that of Fluconazole, it showed better susceptibility results, which is relatively the vital deciding factor of the assay. The details of the obtained results were tabulated [Tables 2-4] for the references. A detailed comparative analysis chart [Figure 1] was prepared for the antimicrobial activities of *Argan* essential oil extract with that of standard antifungal agents against clinical skin scrape isolates of *T. rubrum* for the references. Thus, this study has shown that the phytochemical compounds present in the *Argan* essential

Table 4: Efficacy of *Argan* essential oil extract against clinical skin scrape isolates of *Trichophyton rubrum*

Specimen	*Argan* essential oil extract		
	Disk diffusion	Minimum inhibitory concentration	Minimum fungicidal concentration
Feet	23 mm S	1.25 µ/ml	1.5 µ/ml
Scalp	22 mm S	1.5 µ/ml	1.75 µ/ml
Beard	27 mm S	1.5 µ/ml	1.75 µ/ml
Groin	20 mm S	1.75 µ/ml	2 µ/ml
Hand	25 mm S	1.75 µ/ml	2 µ/ml
Nail	23.5 mm S	2 µ/ml	2.25 µ/ml
Perineum	18.5 mm S	2.25 µ/ml	2.5 µ/ml
Palm	22.5 mm S	2..25 µ/ml	2.5 µ/ml
Finger	26 mm S	2.5 µ/ml	2.75 µ/ml
Average value	23.05 mm S	1.86 µ/ml	2.11 µ/ml

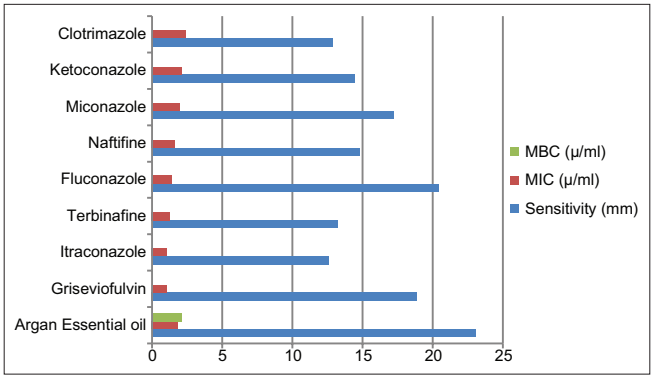

Figure 1: Comparative analysis for the antimicrobial activities of *Argan* essential oil extract versus standard antifungal agents against clinical skin scrape isolates of *Trichophyton rubrum*

oil proves to be more an effective antifungal substance toward the clinical skin scrape isolates of *T. rubrum* compared to the standard antifungal agents.

CONCLUSION

The phytochemical compounds present in the *Argan* essential oil acts as an effective remedy toward the clinical skin scrape isolates of *T. rubrum* compared to the standard antifungal agents. The interpretation of the observation and results for the *Argan* essential oil showed the promising study results regarding its efficacy as potential antifungal agents when compared to that of the standard synthetic chemical agents used against the clinical skin scrape isolates of *T. rubrum*. This study recommends the use of natural essential oils from the plant source as an alternative toward the synthetic chemical antimicrobial substances with more detailed studies need to be done in the near future with the expectations that many dangerous infections can be cured with these types of phytochemical compounds.

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CONTRIBUTION OF AUTHORS

All authors have made substantial contribution to the work and approved it for publication.

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