OPEN ACCESS

Saudi Journal of Pathology and Microbiology

Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) | ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Detection of Antimicrobial Activity of *Acacia nilotica* Extract on Gram-Negative Bacteria Isolated from Clinical Specimens in Shendi Town, Sudan

Leila Mohamed A. Abdelgader[1], Tagreid Hassan Khaleid M. Zain[1], Ghanem Mohammed Mahjaf[1*]

[1]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan

DOI: https://doi.org/10.36348/sjpm.2024.v09i12.001 | **Received:** 28.09.2024 | **Accepted:** 04.11.2024 | **Published:** 05.12.2024

***Corresponding author:** Ghanem Mohammed Mahjaf
Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Shendi, Sudan

Abstract

Background: In the underdeveloped world, microbial infections constitute a serious public health concern. About half of all deaths in tropical nations are thought to be caused by infectious diseases. In addition, some well-known pathogens have assumed new epidemiologic importance by acquiring antimicrobial resistance. Some plants are known as medicinal because they contain active substances that cause specific reactions relating to the cure of human disease. *Acacia nilotica*, commonly known in Sudan as Garad or Sunt, has many medicinal uses. ***Objectives***: This study used the agar diffusion method to study the antibacterial activity of the different concentrations of ethanolic extract of the medicinal plant *Acacia nilotica* pods against selected gram-negative bacteria. ***Methodology***: A cross-sectional study was done in Shendi Town, River Nile State, at the microbiology laboratory at Shendi University. Eighty-five wound swabs and urine specimens were collected from patients with symptoms of urinary tract infection and wound infection who attended Shendi Teaching Hospital and Almek Nimir University Hospital from February to March 2023; bacteria were isolated and identified using colonial morphology, Gram stain, and biochemical tests. Using the agar well diffusion method, we tested the *Acacia nilotica* extract at 100%, 50%, 25%, and 12.5% w/v concentrations. ***Results***: This study found infection was persistent in patients aged 26–34. *Escherichia coli* was a primary isolate. According to gender, out of 85 specimens, 26 (30.6%) were males, whereas 59 (69.4%) were females. The ethanolic extract of *Acacia nilotica* pods was screened for their antimicrobial activity against clinical isolates of *Escherichia coli, K. pneumoniae, Pseudomonas aeruginosa, Enterobacter, Citrobacter, Serratia, Providencia, and Proteus vulgaris*. The antimicrobial effect of the extract was compared with the standard drugs. Crude extracts of *Acacia nilotica* inhibited the growth of various bacteria and showed its broad spectrum antimicrobial potential, which may be employed in managing microbial infections. ***Conclusion***: The fruits of *Acacia nilotica* were the subject of these investigations, demonstrating its potent in vitro activity against clinically isolated bacteria and its potential as a treatment for various bacterial and viral infections.

Keywords: Antimicrobial, Acacia nilotica, Gram-Negative Rods, Herbal Medicine.

1. INTRODUCTION

Even though the primary means of treating bacterial illnesses is through antibiotics, the emergence of antibiotic-resistant bacteria is a global health concern. Antibiotic resistance is rising, and multidrug-resistant strains of microorganisms are emerging due to excessive and repeated antibiotic usage [1]. Planning and observing the impact of focused treatments requires access to data on the scope and patterns of microbial resistance, which can only be obtained through antimicrobial resistance monitoring [2]. Gram-negative rods are among the most significant public health problems in the world due to their high antibiotic resistance. Due to the high risk of morbidity and death associated with these bacteria, patients frequently need to be in the critical care unit, making them clinically significant in hospitals. Two large groups, Enterobacteriaceae and the non-fermenters, are responsible for most clinical isolates [3]. Therefore, developing novel antimicrobial drugs is desperately needed to stop the spread of bacterial resistance. Even new antimicrobial medicines, however, are likely to have a limited therapeutic life due to the quick development of broad resistance [4]. Herbal medicines and natural plant sources may be an overlooked source of novel antibacterial compounds with novel modes of action [5]. In addition to minerals and primary metabolites, plants contain secondary metabolites with antibacterial and

antioxidant properties [6]. According to the World Health Organization, plants are a source of compounds that can combat disease and have antimicrobial, antiviral, and antifungal activities [7]. Some plants are known as medicinal because they contain active substances that cause some reactions when treating human diseases [8]. Some antibiotics have become obsolete because of drug resistance; consequently, new drugs must be sought, so herbal treatment is one possible way to treat diseases caused by multidrug-resistant bacteria. Plant extracts and phytochemicals with known antibacterial properties may be essential in treatments. Several studies have been conducted in different countries to prove such efficiency in the past few years [9]. *Acacia nilotica* is a multipurpose plant used to treat various diseases; the plant contains a profile of multiple bioactive components [10]. *Acacia nilotica* is also known as gum Arabic tree, Babul, and Egyptian thorn, a multipurpose nitrogen-fixing tree legume. It is widely spread in subtropical and tropical Africa, from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India [11]. One of the most significant mediators of inflammation, prostaglandin, is inhibited by anti-inflammatory drugs during its production. Additional mechanisms of anti-inflammatory efficacy include inhibiting certain bradykinin functions and stabilizing lysosomal membrane enzymes in leucocytes, which degrade cartilage and other tissues and prolong inflammation [12]. *Acacia nilotica*, a versatile plant in the world of herbal medicine, has been used to treat various conditions. Herbalists have employed it to address colds, diarrhea, dysentery, inflammation, itch, measles, sore throat, wounds, and sexually transmitted diseases. The plant's antiemetic, antibacterial, expectorant, and antiseptic activities enhance its utility. Its juice, a key component in folk remedies, has even been used to address cancerous conditions. The wide range of applications of *Acacia nilotica* is a testament to its versatility and, more importantly, its potential to inspire new developments in herbal medicine [13, 14]. Antimicrobial resistance is a significant health problem threatening human life worldwide; however, *there is* hope. *Acacia nilotica*, known for centuries for its antimicrobial effect, can be a beacon of light in this dark scenario. It has been used in Sudanese culture for a long time to treat respiratory tract infections, gastrointestinal tract infections, and wound infections. The present study aimed to evaluate the antimicrobial effect of *Acacia nilotica* on gram-negative bacteria isolated from Shendi City, and the results are promising.

2. MATERIALS AND METHODS

This descriptive cross-sectional study, conducted in the unique location of Shendi City, aimed to assess the antimicrobial effect of Acacia nilotica on gram-negative rods. From February to March 2024, Fifty-Eight samples (n=85) were collected at different hospitals and clinical centers in Shendi locality, River Nile State, Sudan. Shendi, a town in Northern Sudan, is situated on the east bank of the Nile (150 km) northeast of Khartoum and about (45 km) southwest of the ancient city of Meroe. Shendi, located in the River Nile state, is the center of the Ja'aliin tribe and an important historic trading center. Its principal suburb on the west bank is Al-Matamma. A prominent traditional trade route across the Bayuda desert connects Al-Matamma to Marawi and Napata (250 km) to the Northwest.

2.1 Collection and Culture of the specimens

Under the aseptic condition, wound swabs were collected using sterile cotton swabs moistened with sterile normal saline, and urine was collected in sterile screw-capped universal containers. To facilitate the identification and isolation of clinical isolates, different types of culture media (CLED agar, blood agar, McConkey agar) were used. The plates were observed for any bacterial colonies to grow significantly. The isolated organisms were fully identified by Gram stain and the appropriate tests, followed by biochemical tests. After identifying isolates, pure culture inoculated with organisms was incubated for 24 hours and preserved in the refrigerator at 4 °C.

2.3 Preparation of serial dilution of Acacia for Testing the Antimicrobial Activity Preparation of extracts

The extraction of the different plants and preservation of the extracts was done according to the method of Obomanu *et al*., [15]. Approximately 25 g of each powdered dried seed was extracted for 72 hours using 100 ml of 95% ethanol in a beaker and later transferred to an airtight bottle to avoid solvent loss. The sample was filtered using muslin cloth and later filter paper. The solvent was removed by evaporation at room temperature. The extract obtained was kept in an airtight bottle until it was used. Acacia was diluted into different concentrations using D.W.: 100%, 75%, 50%, and 25% against the selected organisms. Clinical isolates were isolated from various samples and subcultured. One ml of normal saline was distributed in test tubes and sterilized in an autoclave at 121 °C for 15 minutes. A loopful of purified bacteria was inoculated in sterile normal saline. Inoculum density was compared with the McFarland standard solution.

2.4 Testing of Acacia for antimicrobial activity against standard and clinical isolates

The agar-well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts [16]. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth. In a good light, match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities, viewing against a printed card or sheet of paper is more accessible. Using a sterile

swab, inoculate a plate of Mueller-Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate at approximately 60 to ensure even distribution. With the petri dish lid in place, allow 3–5 minutes for the surface of the agar to dry. Then, a hole with a 6 to 8 mm diameter is punched aseptically with a sterile cork borer (No. 8); Acacia is introduced into the well at the desired concentration [16]. The plates were then incubated upright at 37 °C for 24 h. The plates were observed for the presence of inhibition of bacterial growth, which was indicated by a clear zone around the wells. The size of inhibition zones was measured, and the antibacterial activity was expressed in terms of the average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics Gentamicin, Meropenem, Carbapenem, and Ciprofloxacin. The endpoint of inhibition, where growth starts [17], is a significant finding in our research.

2.5 Standard Antibiotic

Gentamicin, Meropenem, Carbapenem and Ciprofloxacin antibiotic discs used in the present Hi Media Chemicals Ltd, Mumbai procured study, India and stored at 4 °C.

2.6 Data Analysis

Data were entered, checked, and analyzed using Microsoft Excel 2010 and SPSS (Statistical Package of Social Science) soft program version 28.0. Proportional data was presented as frequencies and percentages.

3. RESULTS

The fruits of *Acacia nilotica* were screened for antimicrobial activity against eight Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter*, *Citrobacter*, *Serratia*, *Providencia*, and *Proteus vulgaris*) and The other twenty-four specimens were gram-positive (Excluded). I was using the cup plate agar diffusion method. The extract obtained from the fruits of *Acacia nilotica* exerted pronounced activity against several bacteria strains tested, as indicated by the diameter of growth inhibition zones.

Table 1: The distribution of clinical specimens according to gender

Gender	Frequency	Percent %
Male	26	30.0%
Female	59	70.0 %
Total	**85**	**100.0 %**

Table 2: The distribution of clinical specimens according to the sample age

Age group	Frequency	Percent %
15-25 years	28	33.0%
26-34 years	39	46.0%
35-44 years	15	18.0%
More than 45 years	3	3.0 %
Total	**85**	**100%**

Table 3: The distribution of clinical specimens according to the sample Type

Type of sample	Frequency	Percent %
Urine	48	56.5%
wound swab	37	43.5%
Total	**85**	**100%**

Table 4: The distribution of clinical specimens according to growth and Gram stain

Variables	Urine	Wound swab	Total
Growth	39	26	65
No growth	9	11	20
Gram Positive	14	10	24
Gram Negative	25	16	41

Table 5: The frequency and percentage of isolated organisms

Pathogens	Frequency	Percent %
Escherichia coli	22	53.6%
Klebsiella pneumoniae	3	7.3%
Enterobacter	2	4.8%
Citrobacter	2	4.8%

Pathogens	Frequency	Percent %
Proteus vulgaris	1	2.4%
Serratia marcescens	1	2.4%
Providencia	2	4.8%
Pseudomonas aeruginosa	8	20.0%
Total	**41**	**100**

Table 6: The sensitivity of gram-negative rods bacteria to *Acacia nilotica* at different concentration

Pathogens	Concentration of ethanolic *A. niltotica* extract							
	100%		75%		50%		25%	
	S	R	S	R	S	R	S	R
Escherichia coli	17	5	6	6	2	20	0	22
Klebsiella pneumoniae	2	1	1	1	1	2	0	3
Enterobacter	0	2	0	2	0	2	0	2
Citrobacter	1	1	0	2	1	1	0	2
Proteus vulgaris	0	1	1	0	0	1	0	1
Serratia marcescens	1	0	0	1	0	1	0	1
Providencia	2	0	0	2	0	2	0	2
Pseudomonas aeruginosa	6	2	2	4	4	4	3	5
Percentage	**70.7%**	**29.3%**	**70.7%**	**29.3%**	**19.5%**	**89.5%**	**7.3%**	**92.7%**

Table 7: The comparison of sensitivity between Gram-negative rods bacteria to antibiotics and different concentrations of *A. nilotica* extract

Pathogens	Antibiotics				Different concentrations of *A. nilotica*			
	CN	CIP	CRO	MEM	100%	75%	50%	25%
Escherichia coli	21	14	16	20	17	6	2	0
Klebsiella pneumoniae	2	2	2	2	2	1	1	0
Enterobacter	2	2	2	2	0	0	0	0
Citrobacter	1	1	2	2	1	0	1	0
Proteus vulgaris	1	0	0	1	0	1	0	0
Serratia marcescens	0	0	0	0	1	0	0	0
Providencia	2	0	2	2	2	0	0	0
Pseudomonas aeruginosa	7	4	4	4	6	4	4	3
Total	**36**	**23**	**28**	**33**	**29**	**12**	**8**	**3**

4. DISCUSSION

Sudanese folkloric medicine uniquely merges African, Arabic, and Islamic cultures. Traditional medicine continues to be the most practical means of treating various illnesses and microbiological infections in underprivileged areas. While Sudan accepts traditional medicine, no updated assessment currently focuses on the most common and effective Sudanese medicinal plants [18]. In this study, we attempted to assess the antimicrobial activity of acacia in different concentrations against gram-negative bacteria isolated from clinical specimens. The antibacterial activity was tested using the well diffusion assay, and the acacia was tested at 100%, 75%, 50%, and 25% (v/v) concentrations. Acacia showed the highest activity against *Escherichia coli*, followed by *Pseudomonas aeruginosa*, and the lowest activity was against *Serratia marcescens*, followed by *Proteus vulgaris*. Moreover, the results indicated that the most effective concentration was 100%, and the effect of acacia was decreased dramatically when the concentration of acacia was decreased. The antibiotic disc performs suitable activities against some bacteria; Gentamicin acts well on *E. coli*, followed by *Pseudomonas aeruginosa*, compared with a concentration of 100%. The other bacteria show sensitivity to all antibiotics, with the Gentamicin (87. 8%) high zone of sensitivity followed by Meropenem (83%), Carbapenem (68.3%), and Ciprofloxacin (56%), while the ethanol extract sensitivity in concentration 100 (70.7%), 75 (70.7%),50 (19. 5%), 25 (7.3%). Most samples were taken from the age group 26-34 years (39), the age group 45, and just three samples, so this study found that infection had high frequency in patients aged 26-34 years. Naturally occurring, *Acacia nilotica* is essential to traditional rural and agropastoral systems. The most excellent stretches are found in Sind. It is found in forest regions, roadside ditches, farmlands, agricultural fields, village grazing lands, wastelands, bunds, and along national highways and railway lines in most of India [19]. Our study found that E. coli was the most susceptible of all the tested bacteria, showing extreme inhibitory activity. This finding is in line with the research of Hassan and Asrar in 2022, who also observed the potent activity of *Acacia nilotica* oil against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, with promising

anticandidal potency. It's worth noting that the oil showed weak activity against *Pseudomonas aeruginosa*, which is consistent with their findings [20]. Our results agree with those of Abdelhamed and his colleagues in 2022, who found the antimicrobial activity of *Acacia nilotica*. The methanol extract showed high activity at all concentrations (100%, 50%, 25%, 12%). The results of the antimicrobial investigation show that the distilled water and methanolic extracts inhibited the growth of all microorganisms (Specified by the zone of inhibition). The results provide promising baseline information for the potential use of these crude extracts in drug development programs in the pharmaceutical industries [21]. A study in 2016 showed that The methanolic extract exhibited inhibitory effects against most of the tested microorganisms, with a zone of inhibition ranging from (11-39 mm). The most significant inhibition zone was obtained from the methanolic extract of *Acacia nilotica* (fruits) against the Gram-negative *Salmonella typhi* (39mm) in 100 mg/ml concentration and Gram-positive *Bacillus cereus* (30mm) in 100 mg/ml concentration comparison with Gentamicin (10µg/disc). These studies conducted for *A. nilotica* (fruits) proved to have potent activities against clinically isolated bacteria in vitro and were considered to treat several bacterial and viral infections [22]. In our study, we evaluated the antibacterial activity of the methanol extract of *Acacia nilotica*, and the result indicates that *Acacia nilotica* has activity against the strains tested. This study is compatible with many studies that say *Acacia nilotica* is commonly used to treat eye conditions, open wounds, and dermatological ailments. Acting as much as an antacid, it can also treat digestive problems [23]. *Acacia nilotica* has antibiotic activity, and its aqueous extracts are antibacterial [24]. It has soothing, astringent, and antiseptic properties [23]. The methanolic extract exhibited the highest antimicrobial activity, possibly due to the more robust extraction capacity of the active component responsible for antibacterial activity. It's crucial to consider several factors that can affect or reduce the efficacy of medicinal plants' antimicrobial activity. This begins from the time of plant collection (it's recommended that the collection of plant parts in most cases, but not always should be done after the flowering stage of the plant), the state of plant processing, and the state of plant storage [25]. This knowledge is essential for anyone involved in collecting, processing, or storing medicinal plants.

5. LIMITATIONS

The extraction technique is a critical factor in the antibacterial effectiveness of medicinal plants. Using different test methods, bacterial strains, and sources of antimicrobial samples in various studies makes comparing results challenging. It's essential to conduct thorough research, as each plant is unique, with distinct secondary metabolite types and concentrations contributing to their antibacterial activity.

6. CONCLUSION

In this study, it was concluded that Acacia nilotica possesses a remarkable antimicrobial effect on gram-negative bacteria. Based on this research, the findings showed that acacia, in its most concentrated forms, was very effective against all pathogenic gram-negative bacteria. Additionally, the type of bacterial species affected the antibacterial activity of acacia in concentrations other than 100%, indicating that the antibacterial effect can vary from one strain to another. The results may justify using the Sudanese fruits of Acacia nilotica as antimicrobial therapy in traditional medicine in Sudan and neighboring countries. Additional research on the mechanism of action and other relevant pharmacological studies, including in vivo analysis, medication formulation, and clinical trials, is strongly advised.

7. RECOMMENDATIONS

1. More efforts should be directed toward herbal medicine, and further research should be conducted in this field.
2. Further research to evaluate the active components responsible for the antimicrobial activity of acacia.
3. Efforts should be directed to evaluate the antimicrobial activity and side effects.
4. Examine the methanolic extract of A. nilotica on different isolates.
5. Acacia can be a solution to the antibiotic resistance problem. It can provide a new source of alternative drugs for antibiotics and can help in avoiding the side effects of these antibiotics.

Consent: The patient's written consent has been collect ed.

ETHICAL APPROVAL

The study was approved by the Department of Medical Microbiology in Medical Laboratory Sciences at Shendi University, the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the study area. This study's aims and benefits were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

ACKNOWLEDGMENT

Firstly, the praise be to Allah for providing us with strength to complete this study. Thanks to all staff of Medical Laboratories Sciences, Department of Medic al Microbiology at Shendi University, Shendi-Sudan for helping complete this study.

Sources of Funding

There was no specific grant for this research fr om any funding organization in the public, private, or n

onprofit sectors.

Conflict of Interest: The authors have declared that no competing interests exist.

REFERENCES

1. Harbottle, H., Thakur, S., Zhao, S., & White, D. G. (2006). Genetics of antimicrobial resistance. *Animal biotechnology*, *17*(2), 111-124. doi: 10.1080/1049539060095092.
2. Álvarez-Fernández, E., Alonso-Calleja, C., García-Fernández, C., & Capita, R. (2012). Prevalence and antimicrobial resistance of Salmonella serotypes isolated from poultry in Spain: comparison between 1993 and 2006. *International journal of food microbiology*, *153*(3), 281-287. doi: 10.1016/j.ijfoodmicro.2011.11.011.
3. Ak, O., Batirel, A., Ozer, S., & Čolakoğlu, S. (2011). Nosocomial infections and risk factors in the intensive care unit of a teaching and research hospital: A prospecive cohort study. *Medical science monitor: international medical journal of experimental and clinical research*, *17*(5), 29-34.
4. Coates, A., Hu, Y., Bax, R., & Page, C. (2002). The future challenges facing the development of new antimicrobial drugs. *Nature reviews Drug discovery*, *1*(11), 895-910. doi: 10.1038/nrd940.
5. Braga, L. C., Leite, A. A., Xavier, K. G., Takahashi, J. A., Bemquerer, M. P., Chartone-Souza, E., & Nascimento, A. M. (2005). Synergic interaction between pomegranate extract and antibiotics against Staphylococcus aureus. *Canadian journal of microbiology*, *51*(7), 541-547. doi: 10.1139/w05-022.
6. Akinmoladun, A. C., Ibukun, E. O., Afor, E., Akinrinlola, B. L., Onibon, T. R., Akinboboye, A. O., ... & Farombi, E. O. (2007). Chemical constituents and antioxidant activity of Alstonia boonei. *African journal of biotechnology*, *6*(10), 1197-1201.
7. Abeysinghe, P. D. (2010). Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. *Indian journal of pharmaceutical sciences*, *72*(2), 167.
8. Qa'dan, F., Thewaini, A. J., Ali, D. A., Afifi, R., Elkhawad, A., & Matalka, K. Z. (2005). The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. *The American Journal of Chinese Medicine*, *33*(02), 197-204.
9. Bhattacharjee, I., Chatterjee, S. K., Chatterjee, S., & Chandra, G. (2006). Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria. *Memórias do Instituto Oswaldo Cruz*, *101*(6), 645-648.
10. Singh, B. N., Singh, B. R., Singh, R. L., Prakash, D., Sarma, B. K., & Singh, H. B. (2009). Antioxidant and anti-quorum sensing activities of green pod of Acacia nilotica L. *Food and Chemical Toxicology*, *47*(4), 778-786.
11. Gill, L. S. (2009). Ethnomedicinal uses of plants in Nigeria. University of Benin Press, Benin City, Nigeria. pp.10-30.
12. Tripathi, K. D. (1994). Essentials of Medical Pharmacology, 3rd ed., New Delhi; Jaypee brothers medical Publishers (P) Ltd, 406.
13. Vikrant, A., & Arya, M. L. (2011). A review on anti-inflammatory plant barks. *International Journal of PharmTech Research*, *3*(2), 899-908.
14. Vogel, H. G. (2002). Drug discovery and evolution, 2nd ed, Springer publication, New York, 772-773.
15. Obomanu, F. G., Fekarurhobo, G. K., & Howard, I. C. (2005). Antimicrobial activity of extracts of leaves of Lepidagathis alopecuroides (Vahl). *J. Chem. Soc. Nig*, *30*(1), 33-35.
16. Valgas, C., De Souza, S. M., & Smania, E. F. A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*(2), 360-380.
17. Cheesbrough, M. (2006). District laboratory practice in tropical countries. Second edition. Part 2. p65, 67, 70.
18. Karar, M. G. E., & Kuhnert, N. (2017). Herbal drugs from Sudan: traditional uses and phytoconstituents. *Pharmacognosy reviews*, *11*(22), 83-103.
19. Jhariya, M. K., Bargali, S. S., & Raj, A. (2015). Possibilities and perspectives of agroforestry in Chhattisgarh. In Precious forests-precious earth 2015 Sep 30. Intech Open.
20. Hassan, E. E., & Asrar, A. A. (2022). Comparative Study of Chemical Constituents of the Seeds Extract of Acacia Seyal and Acacia nilotica and its Effect as antimicrobial and Antioxidant, (1), 1-25.
21. Abdalla, A. A., Mustafa, M. I., & Makhawi, A. M. (2020). Phytochemical screening and antimicrobial activities studies of Acacia nilotica fruit cover. *bioRxiv*, 2020-02.
22. Gmaraldeen, S. M., Magzoub, A. A., Badri, A. M., Garbi, M. I., & Saleh, M. S. (2016). Antibacterial activity of Acacia nilotica fruits extract against pathogenic bacteria. *Int J Appl Res*, *2*(6), 103-106.
23. Davidow, J. (1999). Infusions of Healing: A treasury of Mexican-American herbal remedies. Simon and Schuster Inc., 149.
24. Kay, M. A. (1996). Healing with plants in the American and Mexican West. University of Arizona Press.
25. Griggs, J. K., Towers, G. H. N., & Taylor, R. S. L. (2001). The effects of storage on the biological activity of medicinal plants from Nepal. *Journal of ethnopharmacology*, *77*(2-3), 247-252.