PSM Journals

PSM
Biological Research

Research Article 2024 | Volume 9 | Issue 1 | 41-50

Open Access

Article Information

Received: October 10, 2023

Accepted: December 30, 2023

Published: January 31, 2024

Keywords
Goat weed,
Bacteria,
Phytochemical,
Methanol extract,
Aqueous extract.

Authors' Contribution
JIO and PTA conceived and designed the study. BUA and IUM did literature review. All the authors were involved in the write-up, laboratory experiments, and statistical analysis; EMY and MNI revised the paper.

How to cite
Aernan, P.T., Odo, J. I., Ado, B.V., Mende, I.U., Yaji E.M., Iqbal, M.N., 2024. Phytochemical and Antibacterial Assessment of *Ageratum conyzoides* Cultivated in Benue State, Nigeria. PSM Biol. Res., 9(1): 41-50.

***Correspondence**
Joel Inya Odo
Email: odojoel@gmail.com

Phytochemical and Antibacterial Assessment of *Ageratum conyzoides* Cultivated in Benue State, Nigeria

Paulyn Tracy Aernan[1], Joel Inya Odo*[2], Benjamin V. Ado[1], Isaac U. Mende[3], Eunice Mnena Yaji[1], Muhammad Naeem Iqbal[4,5]

[1]Department of Microbiology, Joseph Sarwuan Tarka University, P M B 2373, Makurdi, Benue State, Nigeria.
[2]Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University, P M B 2373, Makurdi, Benue State, Nigeria.
[3]Microbiology Unit, Department of Biological Sciences, Abubakar Tafawa Balewa University, P M B 0248, Bauchi State, Nigeria.
[4]Pacific Science Media, England, United Kingdom;
[5]Association of Applied Biomedical Sciences, Narowal, Pakistan.

Abstract:
The study was conducted to check the phytochemical composition and antibacterial potential of *Ageratum conyzoides* (Goat weed) leaf extracts on selected test bacteria. Samples of fresh leaves were collected and screened for phytochemicals using methanol and aqueous solvents using standard methods. The Agar well diffusion technique was used to assess the antibacterial activity of two extracts (methanol and aqueous) at varying concentrations against the chosen test organisms. Additionally, the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of the extracts were determined. Alkaloids, tannins, flavonoids, saponins, steroids, and phenols were found in both extracts as determined by the phytochemical investigation, however, terpenoids was absent. The statistical analysis indicated a significant difference ($p<0.05$) in the zones of inhibition at different concentrations among the methanol and the aqueous extract of the leaf, with the methanolic extract exhibiting stronger antibacterial activity than the aqueous extract. From the research results, it was found that both aqueous and methanol extracts have the same MIC of 50% for each test bacteria while the MBC for methanol extracts for *E. coli* was 40% and 25% for both *Salmonella* species and *S. aureus* and for aqueous leaf extracts, the MBC for *E. coli* was seen as 25% and for *Salmonella* species 30% except for *S. aureus* where no MBC was observed. The Methanol extract is more effective than the aqueous which may be due to the varying polarity of the solvents, the different phytoconstituents are soluble to varying degrees. The results of the study indicate that more work is necessary to identify and evaluate the effectiveness of the *A. conyzoides* leaf extracts in order to potentially use them for the treatment of certain bacterial illnesses.

Scan QR code to visit this journal.

INTRODUCTION

Plant resources are abundant in Africa, including Nigeria. Unfortunately, because of an over-dependence on Western goods, these natural gifts from God have been overlooked (Abou-Zaid *et al.*, 2021; Onuoha *et al.*, 2013). Many Nigerian communities recognize *Ageratum conyzoides*, often known as goat weed, by different names. The Yorubas call it as *Imiesu;* Igbo, *Ula ujula;* Hausa, Idoma; Utolugwu, Tiv; *Ngokwasw Ahenhen;* and Igedes*, Ufuopioko* (Amadi *et al.*, 2012; Onuoha *et al.*, 2013). It has been documented that about 70–95% of the rural population living in developing nations still mostly rely on medicinal herbs for their first line of treatment (Nasrin, 2013; Prasathkumar *et al.*, 2021; Rahman *et al.*, 2022). Plant extracts have been utilized for a very long period in industrialized nations because they are natural and generally harmless (Iqbal *et al.*, 2019). Phytochemicals, which have anti-oxidant micronutrients like copper and zinc as well as organic and inorganic substances like phenolic acid, are primarily responsible for the therapeutic qualities of plants (Al-Mahweety, 2016; Altemimi *et al.*, 2017; Michalak, 2022; Sharma *et al.*, 2021). Only 15% of medicinal plants have been assessed globally for their phytochemical and phytopharmacological potential (De Luca *et al.*, 2012).

Medicinal plants are abundant in providing biological habitats for dietary supplements, pharmaceutical intermediates, and conventional and contemporary medicine (Iqbal and Ashraf, 2018; Onuoha *et al.*, 2013; Shahzad *et al.*, 2017). The tropical medicinal plant *A. conyzoides* contains pharmacologically active components (Singh *et al.*, 2016). It has been used as a therapy for a variety of ailments in different regions of the world. It is a traditional treatment for craw-craw, boils, wounds, and leprosy in Cameroon, India, and Brazil. It is also used internally for uterine problems, purulent ophthalmia, and gynecological illnesses (Chahal *et al.*, 2021; Dash and Pn, 2011). In addition, the extract is widely used in Nigeria to treat skin conditions and promote wound healing. A decoction of the extract is also administered orally to cure diarrhea and relieve children's navel aches (Kotta *et al.*, 2020; Md *et al.*, 2013; Sharma *et al.*, 2021). Antimicrobial resistance causes several problems, including the inability of antimicrobial medications to treat illnesses caused by infectious organisms (Iqbal and Ashraf, 2020; Osuntokun *et al.*, 2018). The WHO has long urged nations to collaborate with traditional physicians to better identify and capitalize on regions that comparatively provide modern, safe treatments for illnesses with both microbial and non-microbial causes (Dhingra *et al.*, 2020). The medicinal benefits of the weed have been recognized since ancient times when it was used to treat conditions such as burns, wounds, infectious illnesses, arthritis, headaches, dyspnea, pneumonia, inflammatory, asthmatic, spasmodic, and hemostatic effects, stomach problems, gynecological disorders, leprosy, and other skin disorders (Oladejo *et al.*, 2003; Osuntokun *et al.*, 2018).

Over time, infectious illnesses caused by bacteria have become the leading cause of death globally (Bhattarai *et al.*, 2021). Reports show that bacteria have undergone modification that certain antibacterial drugs are now resistant to bacterial infections, as well as the later side effects on humans (Ashraf *et al.*, 2020; Breijyeh *et al.*, 2020; Xuan *et al.*, 2023). The problem of rising antibiotic resistance, together with the variety of adverse effects resulting from current medicines and the rise of illnesses for which there is now no cure, makes the hunt for novel antimicrobial drugs a very pertinent and significant area of study. Plants have long been employed in traditional medicine to treat microbiological diseases (Chinemerem Nwobodo *et al.*, 2022; Iqbal and Ashraf, 2021; Muteeb *et al.*, 2023; Xuan *et al.*, 2023).

It has been suggested that *A. conyzoides* crude extracts provide a potential means of controlling plant-pathogenic fungi (Singh *et al.*, 2013).

There are reports that the leaves of the plant contain anti-inflammatory qualities and don't appear to be harmful to the liver (Moura *et al.*, 2005). *A. conyzoides* essential oil has a distinct aroma and has been shown to have anti-inflammatory, analgesic, and antipyretic properties (Abena *et al.*, 1996; Sukmawan *et al.*, 2021). The current study was carried out to check the phytochemical composition and antibacterial potential of *A. conyzoides* leaf extracts on selected test bacteria.

MATERIAL AND METHODS

Plant samples

The investigation was conducted at the Microbiology Laboratory of Jospeh Sarwuan Tarka University Makurdi, Benue State, Nigeria whereas the fresh samples of *Ageratum conyzoides* were collected and identified in the Botany Department of the University. The leaves were cleaned, sorted, and allowed to air dry in the laboratory for two weeks at room temperature. A mortar and pestle were used to pound the dried leaves into a powder, which was then filtered through a sieve. For a subsequent extraction, a sealed container was subsequently used to store the leaf powder.

Preparation of crude extract

The powdered leaves were weighed (100g), and then steeped for three days (seventy-two hours) in 500ml of distilled water and 500ml of methanol while being continuously shaken. Whatman No. 1 filter paper was used to filter the extracts to get rid of the residue. To get the crude extract, the filtrate was evaporated in a water bath at 50 °C (Idris *et al.*, 2009). The prepared aqueous and methanol extracts were stored in sample containers until needed for phytochemical and antibacterial testing.

Phytochemical Screening

Standard procedures were used to phytochemically screen the aqueous and methanolic leaf extracts of *A. conyzoides* for the incidence of alkaloids, flavonoids, saponins, tannins, steroids, quinones, proteins, glycosides, phenols, and terpenoids (Aernan *et al.*, 2023; Ngbede *et al.*, 2008).

Test for Steroids

A few drops of acetic acid were used to dissolve 1g of the plant extract. After being slowly heated and cooled by running water, a drop of concentrated H_2SO_4 acid was applied to the sidewalls of the test tube. The incidence of steroids was revealed by the emergence of green color (Das *et al.*, 2014).

Test for Saponins

After rapidly shaking around 2ml of the extracts with distilled water, the incidence of saponins was confirmed by the production of foamy leather (Auwal *et al.*, 2014).

Test for Phenol

In a test tube containing 1ml of the extract in 2ml of distilled water, a few drops of 10% ferric chloride solution were added; the green coloration suggested the incidence of phenol (Ahmed *et al.*, 2019).

Test for Quinones

Quinones were detected by a blue-green or red color when around 2ml of the extract was combined with concentrated sulphuric acid (Irum *et al.*, 2021).

Test for Terpenoids

Two milliliters of the extract were mixed with a few drops of chloroform, and to create a layer, a few drops of strong sulfuric acid (H_2SO_4) were added. The existence of terpenoids was quickly confirmed by the production of a reddish-brown precipitate (Irum *et al.*, 2021).

Test for Flavonoids

When two to four drops of ferric chloride were added to 2ml of extract, the presence of flavonoids was indicated by a blackish-red hue (Tyagi and Agarwal, 2017).

Test for Tannins

One milliliter of the extract was mixed with roughly three drops of 0.1% ferric chloride; the outcome was a blue-black or brownish-green color that indicated the occurrence of tannin (Auwal *et al.*, 2014).

Test for Alkaloids

A few drops of 2N HCL were mixed with around 2ml of the extract to create an aqueous layer, which was then decanted and mixed with one or two drops of Mayer's reagent. Alkaloids were present when a white turbidity or precipitate formed (Etangetuk and Idung, 2023).

Antimicrobial Activity Testing

Test Organisms

Escherichia coli, *Salmonella* spp., and *Staphylococcus aureus* cold-stored Agar slant cultures were acquired from the Microbiology Laboratory of the Joseph Tarka Sarwuan University, Makurdi, Benue State. The organisms were resuscitated in buffered peptone broth for a viability test before being subcultured onto nutrient agar medium and incubated at 37°C for 24 hours. By subjecting the cultures to a variety of biochemical tests, such as coagulase production, catalase, indole, citrate utilization, and oxidase test, the likely identity of the clinically derived isolates was further established (Hussain *et al.*, 2016; Lyne and Grange, 1995; Saleem *et al.*, 2018a; Saleem *et al.*, 2018b).

Reconstitution of the plant extracts

After weighing the aqueous and methanolic extracts (1 g) each, they were combined with 2 ml of distilled water and methanol, respectively, to yield a 500 mg/ml concentration. The double broth dilution procedure was then used to create concentrations of 250 mg/ml, 200 mg/ml, 125 mg/ml, and 100 mg/ml (Udochukwu *et al.*, 2015).

Antimicrobial activity of the plant extracts

Sterilized Mueller-Hinton Agar plates were used for the agar well diffusion method of susceptibility testing (Magaldi *et al.*, 2004; Valgas *et al.*, 2007). This approach involved preparing 0.5 MacFarland turbidity standards of *Salmonella* spp., *E. coli*, and *S. aureus* in a standard saline broth for 24 hours. The prepared Muller-Hinton agar was then poured onto a petri dish, stirred to ensure level plating, and allowed to harden. Approximately 0.5ml of each organism from the 24-hour normal saline broth was pipetted onto the dish. The agar plate was surface-drilled with a sterile cork borer (4 mm) to form wells, and the holes were labeled. The extracts were pipetted into the wells using a Pasteur pipette to provide about 0.2 ml of each concentration. The resultant zone of inhibition was kept from overlapping by appropriately spacing the wells. The control was ciprofloxacin at 500 mg/ml. With a millimeter-calibrated ruler, the diameter of the well was determined as the resultant zones of inhibitions in triplicate runs of the experiment.

Minimal Inhibitory Concentration (MIC) of the plant extracts

The macro broth dilution procedure was used to measure the minimum inhibitory concentration (MIC) of the extracts (Aernan *et al.*, 2023; Baron and Finegold, 1990). Four sterile test tubes were used to inoculate a standardized suspension of bacteria into a double-fold serial dilution of the extract in a standard saline broth. The combinations were incubated at 37 °C for 24 hours for bacteria and 72 hours for fungus in sterile test tubes. The mixes were then checked for turbidity, which indicates growth, or absence of it, which indicates inhibition. The extract solution concentration that suppressed microbiological growth was found to be the minimal inhibitory concentration.

Minimum Bactericidal Concentration (MBC) of the plant extracts

The method outlined below was used to calculate the MBC of the corresponding extracts (Amadi *et al.*, 2012; Asowata *et al.*, 2013). An aliquot of the test mixture was removed from the MIC tube that did not exhibit any apparent growth, and it was then subcultured onto a freshly prepared Nutrient agar plate. The plate was prepared following the manufacturer's instructions, and the bacteria were then incubated for 24 hours at 37°C. The lowest concentration of extract that demonstrated no

bacterial growth was identified as the minimal bactericidal concentration.

Statistical analysis

The means and standard deviations of the data were examined using ANOVA, and differences in parameters were assessed for significant differences at $p<0.05$. A statistical package service solution (SPSS) version 21 was used for all of the analysis.

RESULTS

Table 1 shows the phytochemical incidence of alkaloids, flavonoids, phenols, quinone, saponins, steroids, and tannin in the aqueous and methanolic leaf extract of *Ageratum conyzoides* while terpenoids were absent.

Table 1. The incidence of phytochemicals in the methanolic and aqueous leaf extract of *A. conyzoides*.

Phytochemical component	Methanolic extract	Aqueous extract
Alkaloid	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Terpenoids	-	-
Quinones	+	+
Steroids	+	+
Tannins	+	+

Key; Present (+), Absent (-)

The zone of inhibition in (mm) of the methanol leaf extract of *A. conyzoides* is presented in Table 2. *E. coli* has the highest zone of inhibition across the different concentrations followed by *Salmonella* spp. while *S. aureus* has the lowest zone of inhibition among the different concentrations. Statistical analysis showed a significant difference across the different concentrations using ANOVA, where P-value= 0.04.

The zone of inhibition (mm) for the aqueous leaf extract of *A. conyzoides* is displayed in Table 3. *Salmonella* spp. has the highest inhibition zone followed by *E. coli,* and then *S. aureus* still has the lowest inhibition zone just as in the methanol extract case.

Tables 4 and 5 display MIC and MBC of methanol and aqueous leaf extract of *A. conyzoides* on the test bacteria respectively. The MIC of the entire test for methanol extracts was observed at 50% and MBC for *E.coli* was 40% and 25% for both *Salmonella* species and *S. aureus*. MIC was 50% for all the test bacteria with aqueous leaf extract and the MBC for *E. coli* was seen as 25% and for *Salmonella* species 30% except for *S. aureus* where no MBC was observed.

Table 2. Zones of Inhibition (mm) of the methanol leaf extract of *A. conyzoides* on the test bacteria.

Test bacteria	**Zones of inhibition (mm) at different concentrations (mg/ml) of extracts**					**Control**
	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)	31.25 (mg/ml)	Ciprofloxacin
E. coli	21.50±7.07	18.50±3.54	19.00±1.414	16.00±0.00	6.50±9.12	23.00±8.9
Salmonella spp.	21.00±0.01	19.50±7.074	17.00±1.42	15.00±1.41	-	-
S. aureus	16.00±0.00	14.50±7.07	14±0.00	-	-	-

Data are expressed as mean plus or minus standard deviation of replicate plates (P-value < 0.05).

Table 3. Zones of Inhibition (mm) of the aqueous leaf extract of *A. conyzoides* on the test bacteria.

Test bacteria	**Zones of inhibition (mm) at different concentrations (mg/ml) of extracts**					**Control**
	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)	31.25 (mg/ml)	Ciprofloxacin
E. coli	21.50±7.07	19.50±2.12	18.00±2.12	17.00±1.41	-	20.50±17.00
Salmonella spp.	24.00±1.41	22.50±7.07	19.00±1.41	18.00±1.41	-	31,00±20.50
S. aureus	22.50±0.00	19.50±2.12	17.50±7.07	-	0.00	_

Data are expressed as mean plus or minus standard deviation of replicate plates (P-value < 0.05).0

Table 4. MIC of the methanol and aqueous leaf extracts of *A. conyzoides* on the test bacteria.

Test bacteria	Minimum inhibitory concentrations (MIC)	
	Methanol	Aqueous
	MIC	MIC
E. coli	50	50
Salmonella spp.	50	50
S. aureus	50	50

Table 5. MBC of the aqueous leaf extracts of *A. conyzoides* on the test bacteria.

Test bacteria	Minimum bactericidal concentration (MBC) (%age)	
	Methanol	Aqueous
	MBC	MBC
E. coli	40	25
Salmonella spp.	25	30
S. aureus	25	-

DISCUSSION

The phytochemical components of the aqueous and methanol leaf extracts of the plants were as follows: alkaloids, steroids, phenols, saponins, tannins, and flavonoids, were present while terpenoids were absent. This result seems to agree with the previous reports (Iqbal *et al.*, 2019; Kris-Etherton *et al.*, 2002; Surh, 2003) they reported that Phytochemicals can be employed as agents for chemoprevention, which is the use of substances to stop, reverse, or delay tumorigenesis, or as chemotherapeutic drugs. Alkaloids have been used medicinally for around 5000 years. They have anti-malarial, anti-cancer, anti-asthma, and antibacterial pharmacological constituents for humans (Dey *et al.*, 2020; Uzor, 2020). Plant extracts can operate against bacterial strains in a variety of ways, including by damaging enzymes that are necessary for cellular energy generation, the manufacture of structural components, and the inactivation or destruction of genetic information.

This research work checked the in vitro activity of the methanol and aqueous leaf extracts of *A. conyzoides* on some selected test bacteria: *E. coli, Salmonella* species, and *S. aureus*. These bacteria are pathogenic and can cause serious infections such as diarrheal, typhoid fever, strep throat, wound infections, gastroenteritis, and urinary tract infections (Oladejo *et al.*, 2003).

The extracts showed antibacterial effects on the test organisms at different concentrations, this effect is due to the different phytochemical constituents of the plants because the phytochemicals have certain chemicals that inhibit the growth of certain bacteria. There was a significant difference in the zone of inhibition ($P< 0.005$) in both the methanol and aqueous extracts at different given concentrations as reported in several previous studies (Aernan *et al.*, 2023; Ashraf and Iqbal, 2022; Hussain *et al.*, 2016).

It was observed that both aqueous and methanol extracts have the same MIC of 50% for each test organism while the MBC for methanol extracts for the test organism is; *E. coli* 50%, *Salmonella* spp. has 25 and likewise *S. aureus*, while no observable MBC effects was experienced on the aqueous extracts of test organisms. This means that the methanol extract outperforms the aqueous, which might be because the two solvents have distinct polarity and, hence, varying levels of solubility for the different phytoconstituents (Aernan *et al.*, 2023; Kebede and Shibeshi, 2022).

CONCLUSION

The therapeutic qualities of *Ageratum conyzoides* are derived from phytochemicals found in its leaves, including tannin, saponin, alkaloids, phenol, and flavonoids. Antimicrobial activity against the test organisms was demonstrated by the aqueous and methanolic extracts at varying doses. The methanol extract in particular showed the most impact among the extracts, and as such, it may be used as an antibacterial agent to treat infectious pathogenic disorders. In the pharmaceutical industry, *A. conyzoides* can be used to make medications

that treat a range of illnesses and conditions. Antibiotics containing ciprofloxacin may be substituted with the extracts in medical procedures.

ACKNOWLEDGMENT

The authors extend their thanks to the Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria for supporting this work.

CONFLICT OF INTEREST

Authors hereby declare that they have no conflict of interest.

REFERENCES

Abena, A., Ouamba, J., Keita, A., 1996. Anti-inflammatory, analgesic and antipyretic activities of essential oil of *Ageratum conyzoides*. Phytother. Res. (United Kingdom).

Abou-Zaid, A.S., Amusa, J., Alabdulwahab, S., 2021. Natural resources in Africa; a gift or curse: The case of Nigeria. J. Afr. Stud. Dev., 13(2): 23-31.

Aernan, P.T., Odo, J.I., Omeji, J.M., Eunice, Y.M., Iqbal, M.N., 2023. Anti-bacterial Activity of *Moringa oleifera* Seeds against Selected Bacterial Pathogens. PSM Microbiol., 8(3): 82-90.

Ahmed, M., Ji, M., Qin, P., Gu, Z., Liu, Y., Sikandar, A., Iqbal, M., Javeed, A., 2019. Phytochemical screening, total phenolic and flavonoids contents and antioxidant activities of *Citrullus colocynthis* L. and *Cannabis sativa* L. Appl. Ecol. Environ. Res., 17(3).

Al-Mahweety, J.A., 2016. Phytochemical Studies on Medicinal Plants, Dracaenaceae resin, of Socotra Island, Yemen. PSM Biol. Res., 1(2): 62-65.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A., 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel, Switzerland), 6(4).

Amadi, B., Majesty, D., Nnabugwu, A., 2012. Chemical profilesof leaf, stem, root and flower of *Ageratum conyzoides*. Asian J. Plant Sci. Res., 2: 428-432.

Ashraf, A., Iqbal, M.N., 2022. Antibacterial Compounds from Ethanolic Extract of *Scenedesmus obliquus* as Alternatives to Antibiotics. Int. J. Altern. Fuels Energy., 6(1): 12-14.

Ashraf, A., Iqbal, M.N., Iqbal, A., 2020. The Rise of Antibiotic Resistance in Clinical Isolates of Bacteria: An Emerging Public Health Problem. Int. J. Molec. Microbiol., 3(1): 15-17.

Asowata, I., Erhabor, J.O., Idu, M., Timothy, O., Obayagbona, N.O., 2013. Preliminary antimicrobial and phytochemical study of the aqueous, alcholic and chloroform extracts of the leaves of *Napoleonaea vogelli* hook. and planch.(lecythidiaceae). J. Microbiol., Biotechnol. Food Sci., 2(4): 2279-2292.

Auwal, M.S., Saka, S., Mairiga, I.A., Sanda, K.A., Shuaibu, A., Ibrahim, A., 2014. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet. Res. Forum., 5(2): 95-100.

Baron, E., Finegold, S.M., 1990. Methods for testing antimicrobial effectiveness. Diagn. Microbiol., 171-194.

Bhattarai, S., Sharma, B.K., Subedi, N., Ranabhat, S., Baral, M.P., 2021. Burden of Serious Bacterial Infections and Multidrug-Resistant Organisms in an Adult Population of Nepal: A Comparative Analysis of Minimally Invasive Tissue Sampling Informed Mortality Surveillance of Community and Hospital Deaths. Clin. Infect. Dis., 73(Suppl_5): S415-s421.

Breijyeh, Z., Jubeh, B., Karaman, R., 2020. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molec., (Basel, Switzerland), 25(6).

Chahal, R., Nanda, A., Akkol, E.K., Sobarzo-Sánchez, E., Arya, A., Kaushik, D., Dutt, R., Bhardwaj, R., Rahman, M.H., Mittal, V., 2021. *Ageratum conyzoides* L. and Its Secondary Metabolites in the Management of Different Fungal Pathogens. Molec., (Basel, Switzerland), 26(10).

Chinemerem Nwobodo, D., Ugwu, M.C., Oliseloke Anie, C., Al-Ouqaili, M.T.S., Chinedu Ikem, J., Victor Chigozie, U., Saki, M., 2022. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J. Clin. Lab. Anal., 36(9): e24655.

Das, B.K., Al-Amin, M.M., Russel, S.M., Kabir, S., Bhattacherjee, R., Hannan, J.M., 2014. Phytochemical Screening and Evaluation of Analgesic Activity of *Oroxylum indicum*. Indian J. Pharm. Sci., 76(6): 571-5.

Dash, G., Pn, M., 2011. Wound healing effects of *Ageratum conyzoides* Linn. Int. J. Pharma. Bio. Sci., 2: 369-383.

De Luca, V., Salim, V., Atsumi, S.M., Yu, F., 2012. Mining the biodiversity of plants: a revolution in the making. Sci., 336(6089): 1658-1661.

Dey, P., Kundu, A., Kumar, A., Gupta, M., Lee, B.M., Bhakta, T., Dash, S., Kim, H.S., 2020. Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). Recent Adv. Nat. Prod. Analysis. 2020:505-67.

Dhingra, S., Rahman, N.A.A., Peile, E., Rahman, M., Sartelli, M., Hassali, M.A., Islam, T., Islam, S., Haque, M., 2020. Microbial Resistance Movements: An Overview of Global Public Health Threats Posed by Antimicrobial Resistance, and How Best to Counter. Front. Public Health., 8.

Etangetuk, N., Idung, J., 2023. Harnessing the Phytochemical Attributes and Potentials of Gongrenema latifolium Leaf Meal Supplement in the Optimization of biochemical indices in Cultured African catfish (*Clarias garipinus*). J. Surv. Fish. Sci., 10(4S): 1061-1078.

Hussain, F., Kalim, M., Ali, H., Ali, T., Khan, M., Xiao, S., Iqbal, M.N., Ashraf, A., 2016. Antibacterial activities of methanolic extracts of *Datura inoxia*. PSM Microbiol., 1(1): 33-35.

Idris, S., Ndukwe, G., Gimba, C., 2009. Preliminary phytochemical screening and antimicrobial activity of seed extracts of *Persea americana* (avocado pear). Bayero J. Pure Appl. Sci., 2(1): 173-176.

Iqbal, I., Ashraf, A., Iqbal, A., 2019. Plant Essential Oils as Potential Antimicrobials: Present Status and Future Perspectives. PSM Microbiol., 4(3): 71-74.

Iqbal, M.N., Ashraf, A., 2018. Recombinant Protein Production in Plants: Biofactories for Therapeutics. Int. J. Molec. Microbiol., 1(1): 38-39.

Iqbal, M.N., Ashraf, A., 2020. A Review on Bacterial Resistance to Cefepime. Int. J. Molec. Microbiol., 3(1): 18-21.

Iqbal, M.N., Ashraf, A., 2021. Volatile Oils as Green Alternative to Antibiotics in Poultry. PSM Vet. Res., 6(3): 89-91.

Irum, S., Shah, A., Erum, S., Khan, F., Khan, H., Hussain, Z., 2021. Screening of Biotic Compounds and Thin Layer Chromatography of Selected Peach Germplasm of Khyber Pakhtunkhwa, Swat, Pakistan. Agrociencia, 55(4): 136-156.

Kebede, B., Shibeshi, W., 2022. In vitro antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. Vet. Medic. Sci., 8(4): 1802-1815.

Kotta, J.C., Lestari, A.B.S., Candrasari, D.S., Hariono, M., 2020. Medicinal Effect, In Silico Bioactivity Prediction, and

Pharmaceutical Formulation of *Ageratum conyzoides* L.: A Review. Scientifica, 2020: 6420909.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D., 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Medic., 113(9): 71-88.

Lyne, P.M., Grange, J.M., 1995. Collins and Lyne's microbiological methods. Butterworth-Heinemann.

Magaldi, S., Mata-Essayag, S., De Capriles, C.H., Pérez, C., Colella, M., Olaizola, C., Ontiveros, Y., 2004. Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis., 8(1): 39-45.

Md, A.R., Rabeya, S., Rasheda, A., Md, S.I., 2013. Antidiarrheal and antidiabetic effect of ethanol extract of whole *Ageratum conyzoides* L. in albino rat model. Afr. J. Pharm. Pharmacol., 7(23): 1537-1545.

Michalak, M., 2022. Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process. Int. J. Molec. Sci., 23(2): 585.

Moura, A.C., Silva, E.L., Fraga, M.C., Wanderley, A.G., Afiatpour, P., Maia, M.B., 2005. Antiinflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides* L. in rats. Phytomed., 12(1-2): 138-42.

Muteeb, G., Rehman, M.T., Shahwan, M., Aatif, M., 2023. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. Pharm., 16(11): 1615.

Nasrin, F., 2013. Antioxidant and cytotoxic activities of *Ageratum conyzoides* stems. Int. Curr. Pharm. J., 2(2): 33-37.

Ngbede, J., Yakubu, R., Nyam, D., 2008. Phytochemical screening for active compounds in *Canarium schweinfurthii* (Atile) leaves from Jos North, Plateau State, Nigeria. Res. J. Biol. Sci., 3(9): 1076-1078.

Oladejo, O., Imosemi, I., Osuagwu, F., Oyedele, O., Oluwadara, O., Ekpo, O., Aiku, A., Adewoyin, O., Akang, E., 2003. A comparative study of the wound healing properties of honey and *Ageratum conyzoides*. Afr. J. Med. Medic. Sci., 32(2): 193-196.

Onuoha, O.G., Jerome Adekunle, A., Osuagwu, V., Iruolaje, F.O., 2013. Investigation of the antibacterial activity of *Ageratum conyzoides* extract on microorganisms isolated from septic wound. Topclass J. Herb. Medic., 2: 182-188.

Osuntokun, O.T., Jemilaiye, T.A., Yusuf-Babatunde, A., Akele, E., 2018. Antimicrobial properties, phytochemical composition, and phenotypic resistance pattern of selected enteropathogenic microorganism on *Ageratum conyzoides* (Goat weed) leaf extract. International J. Microbiol., 7(2): 18-128.

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., Sadhasivam, S., 2021. Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. Phytomed. Plus., 1(2): 100029.

Rahman, M.H., Roy, B., Chowdhury, G.M., Hasan, A., Saimun, M.S.R., 2022. Medicinal plant sources and traditional healthcare practices of forest-dependent communities in and around Chunati Wildlife Sanctuary in southeastern Bangladesh. Environ. Sustain., 5(2): 207-241.

Saleem, M., Batool, A., Iqbal, M.N., Ashraf, A., 2018a. Characterization of Ceftazidime Resistance in Clinical Isolates of Bacteria in Lahore, Pakistan. Int. J. Molec. Microbiol., 1(2): 44-50.

Saleem, M., Latif, A., Ashraf, A., Iqbal, M.N., 2018b. Characterization of Carbapenem Resistance in Bacteria Isolated from Clinical Samples in Lahore, Pakistan. Int.J. Nanotechnol. Allied Sci., 2(2): 22-27.

Shahzad, M.I., Ashraf, H., Iqbal, M.N., Khanum, A., 2017. Medicinal Evaluation of Common Plants against Mouth Microflora. PSM Microbiol., 2(2): 34-40.

Sharma, T., Tyagi, V., Bansal, M., 2021. Vegetable and Herbal Extracts: A Way towards Preventive and Therapeutics Regimen, Vegetable Crops-Health Benefits and Cultivation. IntechOpen.

Singh, B., Kumar, V., Sinha, D., Agrawal, R., Vadhana, P., Bharadwaj, M., Singh, S., 2016. Antimicrobial activity of methanolic extract and ether extract of *Ageratum conyzoides*. Pharm. Analytica Acta., 7(3): 471.

Singh, S., Devi, W.R., Marina, A., Devi, W.I., Swapana, N., Singh, C.B., 2013. Ethnobotany, phytochemistry and pharmacology of *Ageratum conyzoides* Linn (Asteraceae). J. Medic. Plants Res., 7(8): 371-385.

Sukmawan, Y.P., Anggadiredja, K., Adnyana, I.K., 2021. Anti-Neuropathic Pain Activity of *Ageratum conyzoides* L due to the Essential Oil Components. CNS & neurological disorders drug targets, 20(2): 181-189.

Surh, Y.-J., 2003. Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer, 3(10): 768-780.

Tyagi, T., Agarwal, M., 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. J. Pharmacog. phytochem., 6(1): 195-206.

Udochukwu, U., Omeje, F., Uloma, I., Oseiwe, F., 2015. Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. Am. J. Res. Communic., 3(5): 225-235.

Uzor, P.F., 2020. Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies. Evid.-based Complement. Altern. Med., 2020: 8749083.

Valgas, C., Souza, S.M.d., Smânia, E.F., Smânia Jr, A., 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol., 38: 369-380.

Xuan, J., Feng, W., Wang, J., Wang, R., Zhang, B., Bo, L., Chen, Z.-S., Yang, H., Sun, L., 2023. Antimicrobial peptides for combating drug-resistant bacterial infections. Drug Resist. Updat., 68: 100954.