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EFFECT OF WHOLE PLANT EXTRACT OF AGERATUM CONYZOIDES L. AGAINST SOME BACTERIAL STRAINS

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ABSTRACT
The Ageratum conyzoides L. is an annual aromatic herb in different areas of Sialkot in Punjab. Two bacterial species Escherichia coli and Rhodococcus rhodochorus were used to test antibacterial activity of plant extract of billy goat weed. Muller Hinton nutrient agar medium was used by mixing it with bacterial species. The microdilution technique was used for MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentrations) values. Two extracts i.e. methanolic and aqueous extracts were used. The concentration of 100.0 mg/ml formed 14.0 mm^2 zone of inhibition against Rhodococcus rhodochorus which was greater zone than other concentrations of aqueous extract. The concentration of 100.0 mg/ml formed 17.0 mm^2 zone of inhibition against Rhodococcus rhodochorus which was greater zone than other concentrations of methanolic extract. Escherichia coli formed no any zone of inhibition against both extracts so it was noted as 0.0mm^2. Hence, in both of extracts methanolic extract showed greater effectivity against Rhodococcus rhodochorus i.e. 17.0mm^2 and 14.0mm^2 respectively. Greater inhibition zone mean that plant Ageratum conyzoides L. severely affects the pathogenic bacteria Rhodococcus rhodochorus, so this plant extract may be used as an effective bio-control agent.

Keywords: Plant Extract; Medicinal Plant; Bacterial Strains; Ageratum conyzoides L.

INTRODUCTION
The Ageratum conyzoides L. is an annual aromatic herb in different areas of Sialkot in Punjab. The A. conyzoides belongs to family ‘Asteraceae’. It is a cool seasonal annual weed commonly grows along roadsides and in barren areas (Kumar, 2014). It has worldwide distribution especially in tropical and subtropical regions. It is commonly known as ‘billy goat weed’ or ‘white weed’. They grow abundantly as weed in many countries in the world (Chandrekar et al., 2020).
Ageratum conyzoides L. is a short living herbaceous plant which has height 20-100 cm (Lamsal et al., 2019). Their flowers are present in the form of clusters and appear as a crown which may be dark blue, purple, pink and white. The flower of Ageratum conyzoides does not have any obvious petals and flowers heads are born in dense racemose corymbs inflorescence (Hadidy, 2019). Phylotaxy, the arrangement of leaves of are opposite, simple, serrate, and vary in length from 10-12 cm with a width

of 6-8 cm. Every leaf and axis is covered with small pubis. The species of these flowers can easily grow from seeds in off season.
Seeds may be sown directly in the garden after last frost date. However, their flowering season is very short. The stems are round, mostly green in color and soft hairs are present on them. Roots are shallow, fibrous, dry quickly and become hard. Species of A. conyzoides L. are economically important because they are grown as ornamental plants in homes and gardens. A significant number of chemical compounds have been identified in their flowers. These plants are important as insecticidal, antimicrobial and antifungal agents (Tennyson et al., 2012). The essential oils extracted from leaves of plants possess antidermatophytic compounds, mosquitocidal activities as well as repellency against mosquitoes (Njateng et al., 2010 & Hadidy, 2019).

Billy goat weeds are a source of many biologically active compounds such as, polyphenolic compounds, flavonoids and many others (Rizvi et al., 2014). These plants are an outstanding source of active natural antioxidants and anticancer agents. They are rich in secondary metabolites with biological activity (Okunade, 2002). A. conyzoides sp. has been exploited for their medicinal and insecticidal purposes (Ravindran et al., 2012). These plants are commonly used in treatment of various diseases including cancer due to their bio-active properties, so they also possess anticancer activities. Traditionally, Ageratum sp. is used as antidysenteric in skin and wound diseases, in the treatment of leprosy and purulent ophthalmic (Rizvi et al., 2014). Species of A. conyzoides have allelopathic effects on number of cultivated crops. Their bioactive compounds have inhibitory effect on tomato and mung bean. They are also used in treatment of pneumonia by rubbing them on the chest of the patient. In Ayurveda people also use them as medicinal weed by their use in treatment of various diseases (Okunade, 2002).

Their extracts are widely used in processed food preservation, in pharmaceuticals and natural therapies (i.e. tumor therapy). Decoction or infusion of plants is given in treatment of various stomach diseases such as diarrhea. Leaves are applied to wounds to stop bleeding in villages (Hadidy, 2019). Hence, it is economically and medicinally important in world for its beneficial uses but more clinical studies are needed to introduce the metabolites of white weed. It is necessary to evaluate and introduce their advanced antimicrobial activity at commercial level. Keeping this in view the proposed study was performed with following objectives.

1.1 Objectives of the Study

The objective of this study were following:

1. To prepare the whole plant extract from A. conyzoides
2. To determine the antibacterial activity of A. conyzoides L. whole plant extract against some bacterial species

REVIEW OF LITERATURE

Extract of Ageratum conyzoides was studied in terms of biological active compound. The compounds in the crude extract were quantified and identified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The most important compounds identified in the crude extract were hexadecanoic acid and squalene. The crude extract of billy goat weed was also investigated for their antibacterial properties against various strains of bacteria, such as. Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, and Pseudomonas aeruginosa (Zeeshan et al., 2012).

The novel copper nanoparticles (CuNPs) were synthesized using the aqueous leaf extract of Ageratum conyzoides. The green-synthesized AC-CuNPs exhibit effective dye degradation properties in the presence of daylight. The photocatalytic activity of AC-CuNPs was assessed against the azo dye Congo red (CR). The copper nanoparticles mediated by Ageratum conyzoides (AC-CuNPs) displayed cubic, hexagonal, and rectangular shapes, with an average size of approximately 80 nm.

Tennyson and his co-workers had studied A. conyzoides as a potent source of natural antioxidants. They collected healthy disease free plants of Ageratum conyzoides L. from Javadhu hills, India The research was performed using three distinct solvent extracts from the leaves of A. conyzoides, focusing on antioxidant properties such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals, tested at various concentrations under in vitro conditions. The study resulted that A. conyzoides is a potent source of natural antioxidant (Tennyson et al., 2012). Cypsela of A. conyzoides was examined morphologically under experiment for their anatomy and arrangement. For morphological study, dry mature and softened cypsela was studied under surface study microscope (Talukdar & Mukherjee, 2008). Cross-section from each cypsela was taken from the middle part. Sections were stained with safranin-fast green combination and mounted in Canada balsam (Johansen, 1940). Cypselar shape, type of surface hair, nature of carp podium, phytomelanin deposition, cellular nature of pericarp etc. were taken into consideration for distinguishing the taxa (Talukdar & Mukherjee, 2008). Experiments were conducted to assess the effect of water stress, pH level and light requirement on seed germination of A. conyzoides and the effect of seed burial depth on seedling emergence. Results indicated that species were moderately drought tolerant. The seeds were positively photoblastic because no germination occurred under dark condition. No seedling emerged from seeds placed more than 2 mm deep in soil. It was indicated that those were primarily surface germinating specie (Lamsal et al., 2019).

A. conyzoides L. was utilized for anticancer agents by extracting the compound from their leaves extract. By their experiments they extracted the compound (1, 4-Cyclohexylphenyl) ethanone which exhibit the strong binding to the kinase domain of PKL1 (polo-kinase like) enzyme. PKL1 when over expresses is the reason of cancer in many cells hence ethanone was extracted as anticancer agent of PKL1 enzyme (Rizvi et al., 2014). The leaf extract of A. conyzoides has been studied for its wound healing properties. Flavonoids present in leave extract of A. conyzoides are designated as wound healer (Panicker et al., 2017). Antidiabetic activity of A. conyzoides L. was analyzed by α-amylase enzyme inhibition by the activity of methanolic extract of A. conyzoides. Carbohydrate uptake requires the inhibition of α-amylase, which is important in the digestion of polysaccharides and glycogen. This has been established as a strategy for managing diabetes (Sharma, 2020). The compounds present in A. conyzoides i.e. prococene and agratochromene were studied and designated as responsible for decrease in juvenile hormone. They are recognized as insecticides because of their ability to induce premature metamorphosis, cause sterilization, initiate diapause, and disrupt the production of sex pheromones (Verma et al., 2014).

The flowers and buds of Ageratum conyzoides in different developmental stages were removed, stored in 70% ethanol, embedded in paraffin and sectioned with a thickness of 5-7μm by microtome. Staining was carried out with Eosine and Hematoxylin. Then slides were studied using light microscope and were photographed. Results indicated that in bisexual florets; consist of five stamens with free filaments and apart from each other. Anthers were tetrasporangiated and development of anther wall was of dicotyledonus type and composed of one-layered epidermis, an endothecium, one middle layer and tapetum. Microspore tetrads of A. conyzoides were tetrahedral and pollen grains were three colpated with echinated sculpturing, at the shedding time. Ovule was anatropous, unitegumic and tenuinucellate. Cell division of megaspore mother cells was of both longitudinal and transverse type and resulted to form linear or massive tetrads. The embryo sac development was of the polygonum type. Results of that research showed the presence of formed crystals in anther, the layer endothecium wall anther, epidermal cells of ovary, ovule, style, stigma and petal that was species specific characteristic (Baghaeifar et al., 2022)

Study on acaricidal property of foam soap containing essential oil of Ageratum conyzoides leaves was tested on Rhipicephalus lunulatus. Four doses of the oil (0.00, 0.02, 0.025 and 0.03μl/g) with four replications for each dose were used in vitro. Each replication consisted of 10 ticks in a Petri dish with filter paper impregnated uniformly with the foam soap on the bottom. The same four doses in three

replications were used in vivo. Each replication was made up of 10 naturally ticks infested goats. Results of the study indicated that foam soap containing essential oil of A. conyzoides leaves was toxic to R. lunulatus. The in vitro mortality rate was observed to vary from 0 to 50% on day 8 after treatment with the controls as compared to 95% with the lowest dose (0.02µl/g) on day 8 and 100% with the highest dose (0.03µl/g) on day 3. Meanwhile, the in vivo mortality rate was observed to be 23.4% with the control on day 8 after treatments whereas the highest dose killed 95.1% of the ticks by this day. The foam soap containing essential oil of the plant was 0.0259 and 0.0173µl/g on day 2 after treatment, in the laboratory and on the farm, respectively. That indicated a potentially high efficiency of that medicated soap on parasite (Poam et al., 2005)

Two highly oxygenated flavones were extracted from the aerial parts of Ageratum conyzoides. Their structures were determined as 3′-hydroxy-5,6,7,8,2′,4′,5′-heptamethoxyflavone and 5,3′-dihydroxy-6,7,8,2′,4′,5′-hexamethoxyflavone based on spectral data and chemical degradation. The structure of the latter was verified through X-ray analysis (Quijnao et al., 1980). The chemical composition of the essential oil of Ageratum conyzoides was analyzed using GC and GC/MS techniques. The essential oil was found to contain approximately 50 chemical compounds, with precocene I (23.34%), precocene II (43.99%), and β-caryophyllene (9.18%) identified as the primary components. Other chromene derivatives were also detected at lower concentrations and documented (Chandra et al., 1996).

The juice of Ageratum conyzoides was used in folk medicine as an external wound healing aid for skin injuries. This study was conducted to investigate the effect of A. conyzoides ethanolic extract (ACE) on the expression of aquaporin-3 (AQP3), an integral membrane protein for water and glycerol transport in keratinocytes. ACE increased AQP3 gene expression at the transcriptional level through the p38 MAPK pathway in HaCaT cells. Furthermore, ACE ameliorated suppression of AQP3 expression caused by ultraviolet B (UVB) irradiation. Agerarin (6,7-dimethoxy-2,2-dimethyl-2H-chromene) was identified as the bioactive compound responsible for the up-regulation of AQP3 expression by enhancing the expression of the transcription factor circadian locomotor output cycles kaput (CLOCK). In conclusion, agerarin was a bioactive compound in ACE responsible for CLOCK-mediated AQP3 expression in keratinocytes (Shin et al., 2021).

The essential oil of Ageratum conyzoides was used for insecticidal properties in 2014 by Lu and his coworkers. The aerial parts of the plants were collected to combat booklice, Liposcelis bostrychophila, and to extract any insecticidal components from the oil. The essential oil of A. conyzoides was obtained through hydro distillation and analyzed using GC-MS. A total of 35 components were identified in the essential oil, with the primary constituents being precocene II (62.68%), precocene I (13.21%), and β-caryophyllene (7.92%). Through bioactivity-guided fractionation, precocene II and precocene I was isolated and confirmed as the active compounds. The essential oil demonstrated contact toxicity against L. bostrychophila, and both the essential oil and the isolated compounds exhibited significant repellent activity against this pest. The findings suggest that the essential oil and its constituent compounds hold potential for development as natural insecticides or repellents for managing insects in stored grains (Lu et al., 2014).

In 2016, in vitro technique for propagation of A. conyzoides was investigated. In vitro germinated seeds were used to establish aseptic shoot cultures of several clones. Seedling stem segments bearing 3-4 nodes were placed on Murashige and Skoog (MS) basal medium plus 20g L-1 sucrose, 8.0g L-1 agar to induce axillary shoot development. Axillary shoots sub cultured into the same medium and nodal segments were sectioned and sub cultured to increase the stock of shoot cultures. Shoot cultures of the selected clone were used to accomplish in vitro propagation experiments. The MS medium, augmented with different concentrations of N-6-Benzyl adenine (BA) (0, 0.88, 1.78, 3.55, or 7.1 µ) either singly or in combination with indole-3-acetic acid (IAA) at 5.7 µ, as potential medium for shoot multiplication by single node segments was tested. The highest rate of axillary shoot proliferation was

induced on the medium supplemented with 7.1 µ Benzyl adenine. Explants were divided; sub cultured and continued to proliferate shoots. A proliferation rate of 5.2 shoots per single node explants every four weeks occurred. The highest number of node per axillary shoots after 8-week culture (4.5 nodes) was obtained in the medium with 0.88 µ BA and 5.7µ IAA. The elongated axillary shoots could be in turn sectioned and sub cultured to form an elongated stem again. Five IAA concentrations (0, 1.14, 2.28, 4.57, 5.71µ) were tested to determine the optimum conditions for in vitro rooting of micro shoots. The highest number of roots per micro shoots was obtained with IAA at 2.28µ. Ninety percent of the in vitro rooted plantlets were successfully established in soil. This micro propagation system of A. conyzoides based on axillary shoot development from nodal segments, followed by in vitro rooting, might be used for rapid and efficient mass propagation of improved selections and disease-free germplasm (Ferrara et al., 2016)

The chemical compositions and antimicrobial activity of essential oils derived from Ageratum conyzoides, a member of the Asteraceae family grown in Vietnam, were investigated. The essential oils were isolated through hydro distillation and analyzed using GC and GC/MS. The Minimum Inhibitory Concentration (MIC) and Median Inhibitory Concentration (IC50) values were determined via the microdilution broth susceptibility assay. The primary compound found in the essential oil of Ageratum conyzoides was β-caryophyllene, accounting for 8.9%. The essential oil demonstrated efficacy against Pseudomonas aeruginosa ATCC 27853, exhibiting MIC values of 64.0 µg/ml (IC50, 21.45 µg/ml) and 32.0 µg/ml (IC50, 15.22 µg/ml) (Hoi et al., 2023)

The study was carried out to know the phytochemicals constituent of Ageratum conyzoides leaves and stem. A. conyzoides was evaluated for total saponins, total phenol and other secondary metabolites contents using standard procedures. The study of the leaves and stem revealed the presence of saponins, flavonoids, alkaloids, tannins, phenol and steroids. The leaves of Ageratum conyzoides gave higher composition of tannins, alkaloid, saponin, flavonoid and phenols than the stem. And there was significant relationship between the phytochemicals present in the two plant parts (Anyanele et al., 2022)

The hydro alcoholic extract of whole plant of Ageratum conyzoides was investigated for its anti-diabetic activity at two dose levels on both alloxan induced diabetic rats and normoglycaemic rats. The blood glucose levels were estimated in both alloxan induced hyperglycaemic and normoglycaemic rats. The acclimatized animals were kept fasting for 24 h with water and injected a dose of 120 mg/kg of alloxan monohydrate in normal saline intraperitoneally.

Test extract at 200 and 400 mg/kg and glibenclamide at 2.5 mg/kg was administered. Blood glucose level was estimated at 0h, 1h, 2h, 4h and 8h respectively. Hydro alcoholic extract of A. conyzoides whole plant was found to produce significant ($p<0.01$) reduction in blood glucose concentration between 2-4 hours of administration in both alloxan induced hyper glycaemic and normoglycaemic rats at 400 mg/kg dose. When compared with the reference control glibenclamide, the extract caused noticeable reduction in the blood glucose level in both classes of animals which was demonstrated in above methods. Hence, it was concluded that, Ageratum conyzoides whole plant extract showed good anti-diabetic activity (Reddy et al., 2012)

MATERIALS AND METHODS

3.1 Collection of plant material and Weighing

Ageratum conyzoides L. plants were collected from the North subsidiary area near the village Marakiwal, of Sialkot. The samples were collected from the different places of the collected area. All the samples were placed in plastic envelopes and labeled their names, numbers and dates. These samples were brought in Botany Lab Department of Botany at Govt. Jinnah Islamia Graduate College, Sialkot. After this the material was washed under running tap water and air dry at room temperature away from direct sunlight. These collected plant materials were weighed carefully (Yohannes et al.,

2022). The name and weight of each sample were recorded carefully and all these samples were kept in botany lab of Govt. Jinnah Islamia Graduate College for further study.

Table 3.1 Locations, Numbers and selected samples

Sr. No.	Area of sample collection	No. of total samples	No. of selected samples
1	Marakiwal	10	05
2	Chak Mandar	10	05
3	Kharota Syedan	10	05
4	Bhagal	10	04
5	Chak Sadev	10	04
6	Waryam	10	03
7	Punuwal	10	05
Total		70	31

Fig. 3.1 Collection of plant Ageratum conyzoides L. from Marakiwal, Sialkot

3.2 Authentication of plant species

All the selected herbs were identified by their names and species from Botanist Prof. Javed kamar, head of department of botany and in charge of Jinnah Islamia botanical garden of Govt. Jinnah Islamia College Sialkot. The plant Ageratum conyzoides which is also called “white weed” was identified for next study.

3.3 Extract preparation

For the extraction the dried material was again weighed before crushing/milling by using a mechanical grinder. The powder of root material was taken 100g by using maceration technique. Two dilution processes were used in solvents such as distilled water and methanol (Paulsamy, 2012). The collected material was diluted in these two solvents, 50ml each. Each solution was filtered by using filter paper (Woldeyes et al., 2012). For evaporation filtrates were poured in petri plates and allowed to evaporate for 72hrs in each solvent (Woldeyes et al., 2012).

Fig. 3.2 Preparation of extract

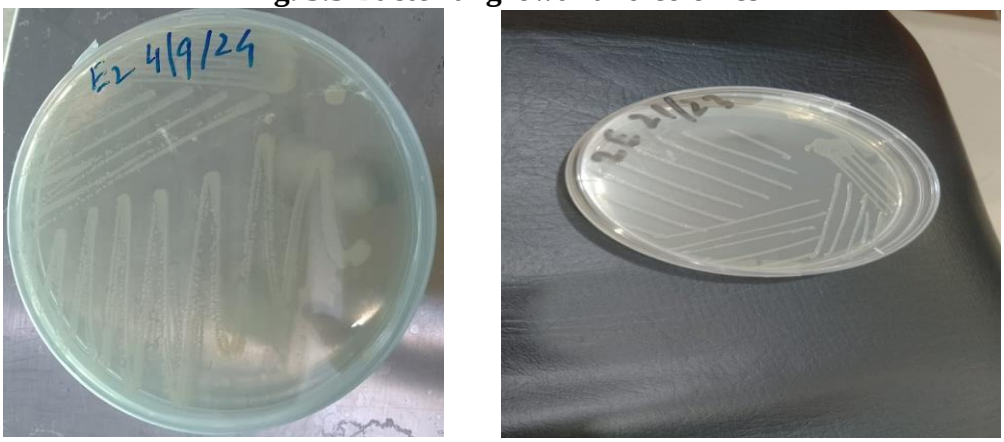
3.4 Scraping and extract collection

When all the solvent was evaporated then the filtrates were scraped with the help of blades for crude extract and collected in the eppendorf tubes (Woldeyes et al., 2012).

3.5 Bacterial species

Bacterial strains Rhodococcus rhodochrous and E. coli were utilized to confirm antibacterial activity. These standard strains were obtained from the Pathology Department of Khawaja Muhammad Safdar Medical College Sialkot (KMSMC). These strains were grown according to their growth requirements on nutrient agar media and used for experimental purposes.

Fig. 3.3 Bacterial growth and colonies



3.6 Preparation of solution

For preparation of solution each extract was formed in various concentrations 1mg/ml, 5mg/ml, 10mg/ml, 50mg/ml and 100mg/ml. Three treatments and three replicated were taken for each concentration. Thus for solution preparation extracts were dissolved in distilled water as in given concentrations.

Table 3.2 Formation of different concentrations from two different solvents

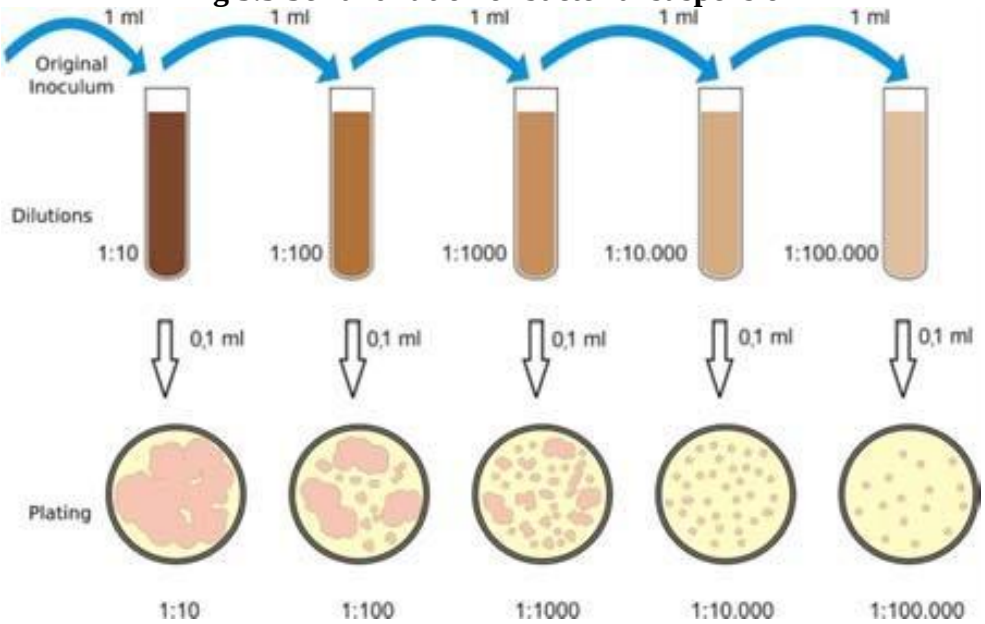
Extract	Methanol	Distilled water	Strength(mg/ml)
5mg	5ml	5ml	1mg/ml
25mg	5ml	5ml	5mg/ml
125mg	5ml	5ml	10mg/ml
250mg	5ml	5ml	50mg/ml
500mg	5ml	5ml	100mg/ml

Fig 3.4 Preparation of solution

3.7 Bacterial suspension preparation

Bacterial suspensions were made in serial dilutions so that at least one of the dilutions contained a suitable concentration of cells. 1ml of bacterial sample was dissolved in 9ml distilled water. This was then followed by the same procedure, where 1ml from tube 1 was added to 9ml distilled water of tube 2 and after this 1ml of this suspension was taken with 9ml of tube 3 and so on the procedure repeated for each tube. Then 0.1ml from each suspension was poured into Nutrient agar media for bacterial growth and incubated at 37°C for 24hrs (Woldeyes et al., 2012).

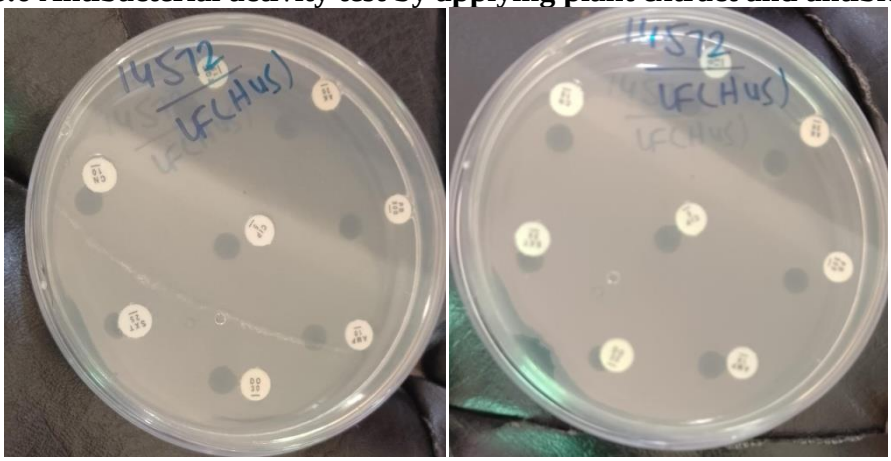
Fig 3.5 Serial dilution of bacterial suspension



3.8 Determination for antibacterial activity

For antibacterial activity test Muller Hinton Nutrient agar medium was used. This agar was prepared by mixing the nutrient agar solution with bacterial stock solution taken from tube 5 with bacterial colony 1×10^5. 100ml of nutrient agar solution was mixed with 1ml of bacterial suspension. 20ml of solution was poured in each petri plate and allow solidifying. Five wells were made in the medium with the help of sterilized cock borer no.8.Then each pore was filled with prepared extracts with the help of micropipette. Extracts were allowed to diffuse at room temperature and incubated at 37°C for 24 hours. Growth of both species was also determined by taking a control against both of them. Azithromycin an antibiotic was taken and applied as a control against both species to determine which specie was affected by control (Egorov, 1985).

Fig 3.6 Antibacterial activity test by applying plant extract and antibiotics



3.9 Evaluation of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration)

The Minimum Inhibitory Concentrations (MIC) was determined by micro dilution technique (Veiga et al., 2019). Different concentrations of plant extracts (mg/ml) were mixed with nutrient agar. Concentrations were mixed with nutrient broth and 100ml of bacterial suspension inoculated and incubated at 37°C for 24 hours. The lowest concentration which completely inhibits the bacterial growth was noted as MIC value. For Minimum Bactericidal Concentrations (MBC) serial dilutions were spread on agar medium and incubated at 37°C for 24-48hrs for viable counts (Ferrazzano et al., 2017).

3.10 Statistical analysis

Data authentication was done by using SPSS software. All the experiments were carried out at different concentrations, each with three replicates and the entire experiment was repeated thrice.

RESULTS

Several results were determined from plant extract of Ageratum conyzoides which showed the antibacterial activity. These results are evaluated by experiments which are done by using plant extract of A. conyzoides.

4.1. Evaluation of antibacterial activity by using methanolic extracts of plant Ageratum conyzoides L.

The methanolic extract concentration against bacteria R. rhodochorus and E. coli had different zones of inhibition with Azithromycin an antibiotic was used for control. The zones of inhibition of R. rhodochorus with methanolic extract concentrations were as 1.0 mg/ ml had 0.0 mm^2, 5.0 mg/ml had 0.0 mm^2, 10.0 mg/ml had 5.0 mm^2, 50.0 mg/ml had 9.0 mm^2 and 100.0 mg/ml had 17.0 mm^2 zones of inhibition. Rhodococcus rhodochorus formed zone of inhibition 19.0 mm with control. The concentration of 100.0 mg/ml had formed 17.0 mm^2 zone of inhibition which was larger zone than other concentrations of methanolic extract. Methanolic extract has no any effect on E.coli and its remains unaffected by methanolic extract. No any zone of inhibition of E. coli was formed. Data is shown in table 4.1 and 4.2.

Fig 4.1 (a) E.coli shows no zone of inhibition while (b) Rhodococcus rhodochorus shows zone of inhibition

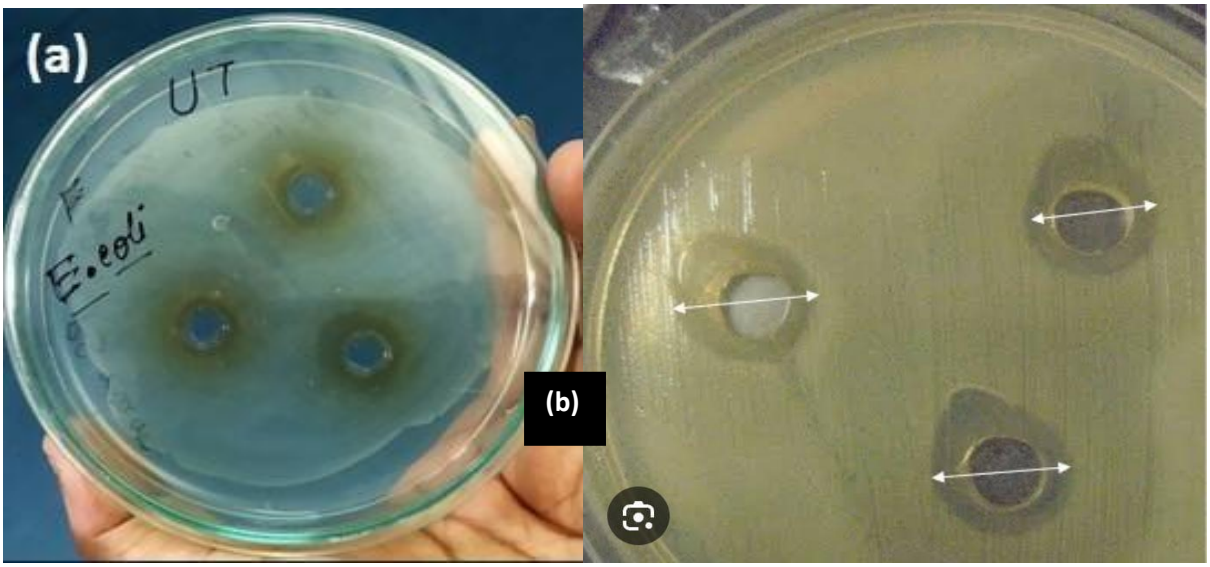


Table 4.1 Effect of methanolic extract concentration on bacterial species

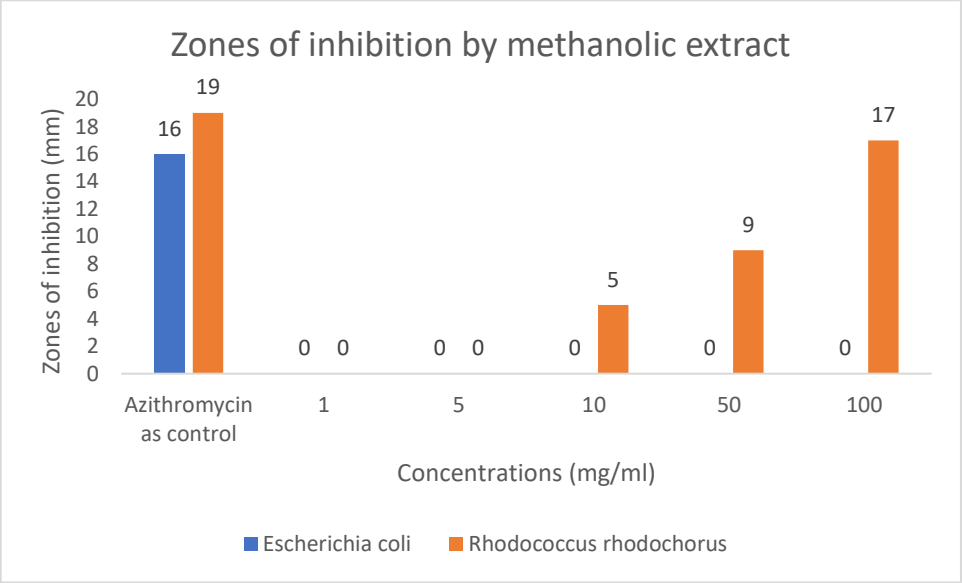
Concentrations (mg/ml)	**Treatment**	**Replication**	**Zones of inhibition (mm^2)**	
1mg/ml			R. rhodochorus	E.coli
	1	1	0	0
	1	2	0	0
	1	3	0	0
			$0mm^2$	$0mm^2$
5mg/ml	1	1	0	0
	1	2	0	0
	1	3	0	0
			$0mm^2$	$0mm^2$
10mg/ml	1	1	4	0
	1	2	5	0
	1	3	6	0
			$5mm^2$	$0mm^2$
50mg/ml	1	1	8	0
	1	2	9	0
	1	3	10	0
			$9mm^2$	$0mm^2$
100mg/ml	1	1	15	0
	1	2	17	0
	1	3	19	0
			$17mm^2$	$0mm^2$

Most effective concentration = 100mg/ml
Moderate effective concentration = 50mg/ml
Less effective concentration = 10mg/ml

Table 4.2 Mean results of methanolic extract against two bacterial species while taking Azithromycin as control

Methanolic concentrations	Bacterial zones of inhibition(mm^2) Azithromycin as control			
	R. rhodochorus	Azithromycin	E. Coli	Azithromycin
1mg/ml	0 mm^2	19 mm^2	0 mm^2	16 mm^2
5mg/ml	0 mm^2		0 mm^2	
10mg/ml	5 mm^2		0 mm^2	
50mg/ml	9 mm^2		0 mm^2	
100mg/ml	17 mm^2		0 mm^2	

Fig 4.2 Zones of inhibition formed by bacterial species in methanolic extract of Ageratum conyzoides L.

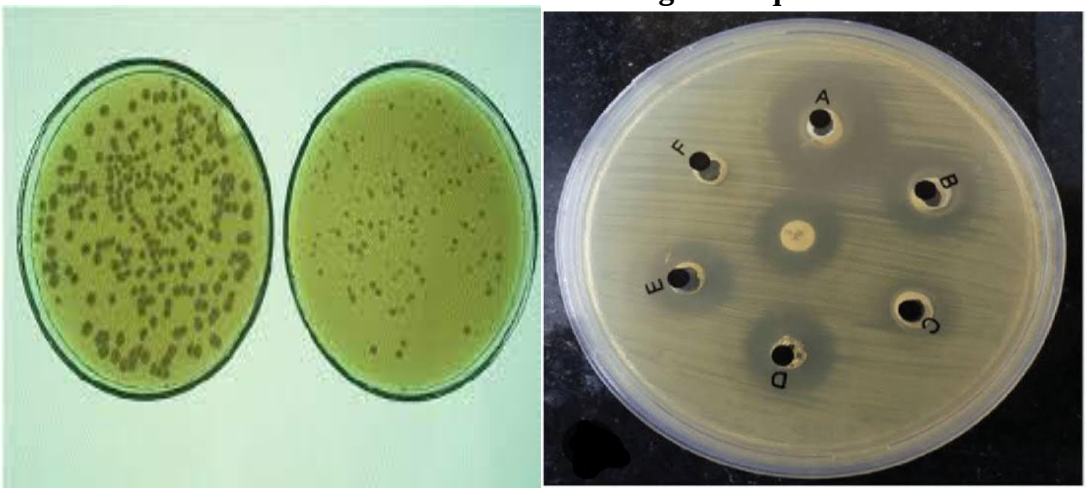


Note: Methanolic extract showed greater zone of inhibition (17.0mm^2) against R. rhodochorus at higher concentration (100mg/ml), while E.coli has not affected by methanolic extract concentration of A. conyzoides hence, no zone of inhibitions are formed. Azithromycin affected both species.

4.2. Evaluation of antibacterial activity by using aqueous extracts of plant Ageratum conyzoides L.

The aqueous extract concentration against bacteria Rhodococcus rhodochorus and Escherichia coli had different zones of inhibition with Azithromycin an antibiotic was used for control. The zones of inhibition of Rhodococcus rhodochorus with aqueous extract concentrations were as 1.0 mg/ ml had 0.0 mm^2, 5.0 mg/ml had 3.0 mm^2, 10.0 mg/ml had 7.0 mm^2, 50.0 mg/ml had 11.0 mm^2 and 100.0 mg/ml had 14.0 mm^2 zones of inhibition. With control Rhodococcus rhodochorus formed zone of inhibition 19.0 mm^2. The concentration of 100.0 mg/ml had formed 14.0 mm^2 zone of inhibition which was larger zone than other concentrations of aqueous extract. E.coli remains unaffected with aqueous extract; no any zone of inhibition was formed by aqueous extract. Hence aqueous extract of plant Ageratum conyzoides was affected against R. rhodochorus but not against E.coli. Data for aqueous extract is shown in table 4.3 and 4.3.

Fig 4.3 (a) E.coli shows no zone of inhibition against aqueous extract, (b) Rhodococcus rhodochorus shows zone of inhibition against aqueous extract



(a) (b)

Table 4.3 Effect of aqueous extract concentration on two bacterial species

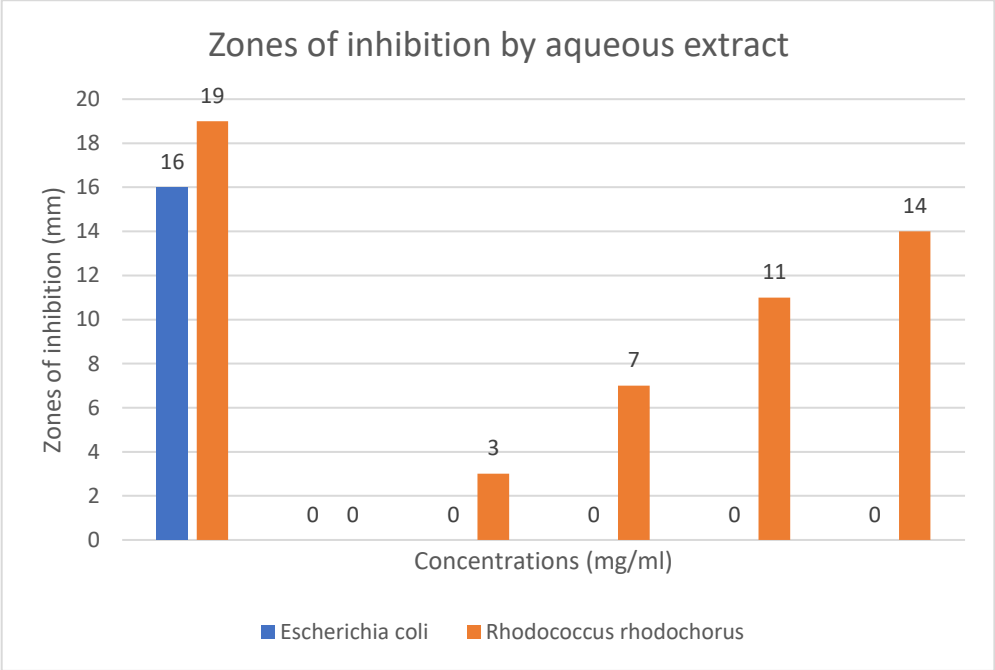
Concentrations (mg/ml)	Treatment	Replication	Zones of inhibition (mm^2)	
1mg/ml			R. rhodochorus	E.coli
	1	1	0	0
	1	2	0	0
	1	3	0	0
			$0mm^2$	$0mm^2$
5mg/ml	1	1	3	0
	1	2	2	0
	1	3	4	0
			$3mm^2$	$0mm^2$
10mg/ml	1	1	5	0
	1	2	7	0
	1	3	9	0
			$7mm^2$	$0mm^2$
50mg/ml	1	1	8	0
	1	2	11	0
	1	3	14	0
			$11mm^2$	$0mm^2$
100mg/ml	1	1	13	0
	1	2	14	0
	1	3	15	0
			$14mm^2$	$0mm^2$

Most effective concentration = 100mg/ml
Moderate effective concentration = 10mg/ml
Less effective concentration = 5mg/ml

Table 4.4 Mean results of aqueous extract concentrations affected two bacterial species while taking Azithromycin as control

Aqueous extract solution	Zones of inhibition(mm^2)			
	R. rhodochorus	Azithromycin	E. Coli	Azithromycin
1mg/ml	0 mm^2	19 mm^2	0 mm^2	16 mm^2
5mg/ml	3 mm^2		0 mm^2	
10mg/ml	7 mm^2		0 mm^2	
50mg/ml	11 mm^2		0 mm^2	
100mg/ml	14 mm^2		0 mm^2	

Fig 4.4 Zones of inhibition formed by bacterial species in aqueous extract of Ageratum conyzoides L.



Note: Aqueous extract showed greater zone of inhibition (14.0mm^2) against R. rhodochorus at higher concentration (100mg/ml), while E.coli has not affected by aqueous extract concentration of A. conyzoides hence, no zone of inhibitions are formed. Azithromycin affected both species

4.3. Evaluation of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentrations (MIC) which inhibits the growth of Rhodococcus rhodochorus was at 5mg/ml which was 3.0mm^2 and value of Minimum Bactericidal Concentration (MBC) was determined at concentration of 10mg/ml for the aqueous extract which was 7mm^2, MIC for the methanolic extract was at 10mg/ml and recorded as 5mm^2 the value of MBC was at 50mg/ml and was 9mm^2 while E.coli remains resistant for both extracts i.e. aqueous and methanolic (Veiga et al., 2019).

Fig 4.5 Inhibitory zone after applying plant extract and antibiotic

4.4 Statistical analysis

All the experiments were carried out at different concentrations, each with three replicates and the entire experiment was repeated thrice and data were analyzed by using SPSS software.

4.4.1 Methanolic extract analysis

Table 4.5(a) Mean Analysis for methanolic extract concentration

Extraction type	N	Mean	Std. Deviation	Std. Error Mean
R. rhodochorus	5	6.20	7.120	3.184
E.coli	5	.00	.000	.000

Table 4.5(b) Independent test for methanolic extract concentration

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	9.882	.014	2.313	8	.049	6.800	2.939	.022	13.578
Equal variances not assumed			2.313	4.000	.082	6.800	2.939	-1.361	14.961

Null hypothesis: There is insignificant difference between average zones of inhibition for R. rhodochorus and E.coli

Alternative hypothesis: there is significant difference between average zones of inhibition for R. rhodochorus and E. coli against methanolic extract.

Result: According to above table the p value for independent samples test is 0.049, which is smaller than 0.05, so the null hypothesis is rejected and it is concluded that there is significant difference between average zones of inhibition for R. rhodochorus and E. coli against methanolic extract.

4.4.2 Aqueous extract analysis

Table 4.6(a) Mean analysis of aqueous extract concentration

Group Statistics

Zone of Inhibition	N	Mean	Std. Deviation	Std. Error Mean
R.rhodochorus	5	7.20	5.404	2.417
E.coli	5	.00	.000	.000

Table 4.6(b) Independent test for aqueous extract concentration

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	13.360	.006	2.979	8	.018	7.200	2.417	1.627	12.773
Equal variances not assumed			2.979	4.000	.041	7.200	2.417	.490	13.910

Null hypothesis: If there is insignificant difference between average zones of inhibition for R. rhodochorus and E.coli

Alternative hypothesis: There is significant difference between average zones of inhibition for R. rhodochorus and E. coli against aqueous extract.

Result: According to above table the p value for independent samples test is 0.18, which is smaller than 0.05, so the null hypothesis is rejected and it is concluded that there is significant difference between average zones of inhibition for R. rhodochorus and E. coli against aqueous extract.

DISCUSSION

Ageratum conyzoides is an annual herbaceous plant with a long history of traditional medicinal uses in several countries in the world and also has bioactivity with insecticidal and antimicrobial activity. The plant Ageratum conyzoides may be an effective bio- control agent. This article was on the antimicrobial activity of Ageratum conyzoides. For determination of antimicrobial activity of A. conyzoides, two dilutions were used with different concentrations and replicate of concentrations with extract of plant Ageratum conyzoides. These two dilutions were methanolic extract and aqueous extract of plant A. conyzoides which showed effectivity against bacterial species. Both methanolic extract concentration and aqueous extract concentration showed their activity against R. rhodochorus. Zones of inhibition formed by methanolic extract against R. rhodochorus were 0.0mm^2, 0.0 mm^2, 5.0 mm^2, 9.0 mm^2 and 17.0 mm^2 with various concentrations of extract. Higher concentration shows greater zone of inhibition i.e. 100mg/ml showed 17.0 mm^2.Zones of inhibition for aqueous extract were 0.0 mm^2, 3.0 mm^2, 7.0 mm^2, 11.0 $mm^2$and 14.0 mm^2 with various concentrations and replicate of those concentrations. Higher concentration showed greater zone of inhibition i.e. 100mg/ml showed 14.0mm^2. Methanolic extract showed greater effectivity than aqueous extract. These results were correlated with the results of Kurade et al., 2010. Earlier work on antibacterial activity of Ageratum conyzoides was done by Kurade and his coworkers in 2010 and they determine the effect of essential oil of A. conyzoides on different bacterial strains. Growth of other strains was not inhibited but Rhodococcus rhodochorus was affected by Ageratum conyzoides L.

MIC and MBC values were higher against methanolic extract than aqueous extract i.e. the Minimum Inhibitory Concentrations (MIC) which inhibits the growth of Rhodococcus rhodochorus was at 5mg/ml which was 3.0mm^2 and value of Minimum Bactericidal Concentration (MBC) was determined at concentration of 10mg/ml for the aqueous extract which was 7mm^2, MIC for the methanolic extract was at 10mg/ml and recorded as 5mm^2 the value of MBC was at 50mg/ml and was 9mm^2 while E.coli remains resistant for both extracts i.e. aqueous and methanolic. The discussion on MIC and MBC was made by Veiga et al., 2019.

Earlier work on antibacterial activity of Ageratum conyzoides was done by Bhattacherjee and coworkers in which growth of E.coli was not inhibited by its means Ageratum conyzoides has no any effect on growth of E.coli (Bhattacherjee et al., 2005). The report on antibacterial activity of Ageratum conyzoides was made by Tedonkeng Pamo and his coworkers in which A. conyzoides has been reported to have an acaricidal activity against Rhipicephalus lunulatus (Tedonkeng Pamo et al., 2005). Pereira in 1929, cited by Jaccoud (1961), reported use of the leaves as an insect (moth) repellent. The insecticide activity may be the most important biological activity of this species. Assays conducted in Colombia by Gonzalez et al. (1991) showed activity of this species against Musca domestical larvae, using whole plant hexane extract. Vyas and Mulchandani (1980) reported the action of cromenes (precocenes I and II), isolated from Ageratum plants, which accelerate larval metamorphosis, resulted in juvenile forms or weak and small adults.

Ekundayo et al. (1987) also demonstrated the juvenilizing hormonal action of precocene I and II in insects, the most common effect being precocious metamorphosis, producing sterile or dying adults. Raja et al., 1987, used A. conyzoides methanolic extract from fresh leaves (250 and 500 ppm) in the fourth instar of Chilo partellus (Lepidoptera, Pyralidae), a sorghum pest, observed the presence of a dark stain in the insects cuticle and immature pupae formation, both symptoms of deficiency of

juvenile hormone. The antimicrobial activity of essential oils from Ageratum conyzoides of was also studied by Hoi and his team in 2023. The essential oil was effective against Pseudomonas aeruginosa ATCC 27853 with MIC values of 64.0 μg/ml) and 32.0 μg/ml (Hoi et al., 2023).

CONCLUSION

The bacterium Rhodococcus rhodochorus had larger zone of inhibition than Escherichia coli. The zone of inhibition was formed methanolic extract of plant Ageratum conyzoides, aqueous extract also affects the Rhodococcus rhodochorus but zones of inhibition were smaller than methanolic extract. Escherichia coli remain resistant against both extract. Hence, larger inhibition zone mean that plant Ageratum conyzoides L. (white weed) has severely affected the pathogenic bacteria Rhodococcus rhodochorus, so the plant extract may be used as an effective bio-control agent.

REFRENCES

1. Anyanele, W. C., Anyanele, S. I., & Ejidike, C. S. (2022). Comparative Study on the Phytochemical Analysis of the Leaves and Stem of Ageratum conyzoides. Asian Plant Research Journal, 9(2), 49-55.
2. Baghaeifar, Z., Hosseininiya, E., Mehrafza, A., Khodaei, L., & Ghadirpour, F. (2022). The Study of Microgametophyte and Megagametophyte Development in Ageratum conyzoides L. Journal of Plant Research, 35(3), 649-663.
3. Bhattacharjee I, Ghosh A, Chandra G (2005): Antimicrobial activity of the essential oil of Cestrum diurnum (L.). African Journal Biotechnology 4: 371–374.
4. Chandra, S., Shahi, A. K., Dutt, P., & Tava, A. (1996). Essential oil composition of Ageratum conyzoides L. from Jammu region of India. Journal of Essential Oil Research, 8(2), 129-134.
5. Chandraker, S. K., Lal, M., Ghosh, M. K., Tiwari, V., Ghorai, T. K., & Shukla, R.(2020). Green synthesis of copper nanoparticles using leaf extract of Ageratum houstonianum Mill. and study of their photocatalytic and antibacterial activities. Nano Express, 1(1), 010033.
6. Egorov, N. S. (1985). Antibiotics: A scientific approach. Mir publishers, Russia, (pp. 440).
7. Ferrara, G., Iapichino, G., Airò, M., Sabatino, L., & Germanà, M. A. (2016, March). Micropropagation of Ageratum conyzoides by nodal segments. In IX International Symposium on In Vitro Culture and Horticultural Breeding 1187 (pp. 29-36).
8. Ferrazzano, G. F., Scioscia, E., Sateriale, D., Pastore, G., Colicchio, R., Pagliuca, C., ...& Pagliarulo, C. (2017). In vitro antibacterial activity of pomegranate juice and peel extracts on cariogenic bacteria. Bio-Medical Research International, 10(1), 1143-1155
9. Hadidy, D., El Sayed, A. M., El Tantawy, M., & El Alfy, T. (2019). Phytochemical analysis and biological activities of essential oils of the leaves and flowers of Ageratum conyzoides L. cultivated in Egypt. Journal of Essential Oil Bearing Plants, 22(5), 1241-1251.
10. Hoi, T. M., Hung, N. H., Huong, L. T., Hau, D. V., Duyen, D. T. H., Setzer, W. N., & Ogunwande, I. A. (2023). Chemical Compositions of Essential Oils and Antimicrobial Activities of Asteraceae: The Leaves of Blumea lacera (Burm. f.) DC., Tridax procumbens (L.), and Ageratum conyzoides., from Vietnam. Journal of Essential Oil Bearing Plants, 26(1), 130-142.
11. Kumar N. (2014), Biological potential of a weed Ageratum conyzoides L.: A review Indo. American Journal Pharmaceutical Research 4(6):2683
12. Kurade, N. P., Jaitak, V., Kaul, V. K., & Sharma, O. P. (2010). Chemical composition and antibacterial activity of essential oils of Lantana camara, Ageratum conyzoides and Eupatorium adenophorum. Pharmaceutical Biology, 48(5), 539-544.
13. Lamsal, A., Devkota, M. P., Shrestha, D. S., Joshi, S., & Shrestha, A. (2019). Seed germination ecology of Ageratum conyzoides L.: A major invasive weed in Nepal. Public Library of Science One, 14(11), e0225430.
14. Lu, X. N., Liu, X. C., Liu, Q. Z., & Liu, Z. L. (2014). Isolation of Insecticidal constituents from the essential oil of Ageratum conyzoides against Liposcelis bostrychophila L. Journal of Chemistry, 2014, 1-6.

15. Ming, L. C. (1999). Ageratum conyzoides: A tropical source of medicinal and agricultural products. Perspectives on new crops and new uses, 1988, 469-473.
16. Njateng, G. S. S., Kuiate, J. R., Gatsing, D., Tamokou, J. D., Mouokeu, R. S., & Kuete, V. (2010). Antidermatophytic activity and dermal toxicity of essential oil from the leaves of Ageratum conyzoides (Asteraceae). Journal of Biological Sciences, 10(5), 448-454.
17. Okunade, A. L. (2002). Ageratum conyzoides L. (Asteraceae). Fitoterapia, 73(1), 1-16.
18. Pamo, E. T., Tendonkeng, F., Kana, J. R., Payne, V. K., Boukila, B., Lemoufouet, J., ... & Nanda, A. S. (2005). A study of the acaricidal properties of an essential oil extracted from the leaves of Ageratum conyzoides. Veterinary Parasitology, 128(3-4), 319-323.
19. Panicker, V. P., Haridas, P., Jayavardhanan, K. K., & John, B.(2017). Evaluation of wound healing properties of methanolic extract of Ageratum conyzoides L. Journal of Livestock Science 8, 201-203.
20. Paulsamy, S., &Karthika, K.(2012). Screening of in vitro antioxidant activity of methanolic leaf and root extracts of Hypochaeris radicata L. (Asteraceae). Journal of Applied Pharmaceutical Science, 2(7), 149-154.
21. Quijano, L., Calderon, J. S., Gomez, F., & Rios, T. (1980). Two polymethoxyflavones from Ageratum conyzoides. Phytochemistry, 21(12), 2965-2967.
22. Ravindran J., Samuel T., Alex E., William J. (2012), Adulticidal activity of Ageratum conyzoides Linn. Leaf extracts against three vector mosquito species. Asian Pacific Journal of Tropical Disease, 2(3):177
23. Reddy, S., Kumar, S., & Ganapaty, S. (2012). Evaluation of Ageratum conyzoides Whole Plant for its Anti-Diabetic Activity. Journal of Advanced Scientific Research, 3(02), 67-70.
24. Rizvi, S. M. D., Shakil, S., Zeeshan, M., Khan, M. S., Shaikh, S., Biswas, D., & Kamal, M. A. (2014). An enzoinformatics study targeting polo-like kinases-1 enzyme: Comparative assessment of anticancer potential of compounds isolated from leaves of Ageratum conyzoides. Pharmacognosy Magazine, 10(1), 14.
25. Sharma K.R., (2020), In-vitro antioxidant, antidiabetic and toxic effect of Ageratum conyzoides from Chitwan district Nepal. Journal of Balkumari College. 9(1): 48-54
26. Shin, S. Y., Koh, D., Lim, Y., & Lee, Y. H. (2021). Inhibition of EGR-1-dependent MMP1 transcription by ethanol extract of Ageratum conyzoides in HaCaT keratinocytes. Molecular Biology Reports, 48(1), 1-11.
27. Singh, V., Singh, H., Sharma, G. P., & Raghubanshi, A. S. (2011). Eco-physiological performance of two invasive weed congeners (Ageratum conyzoides L. and Ageratum houstonianum Mill.) in the Indo-Gangetic plains of India. Environmental Monitoring and Assessment, 178, 415-422.
28. Talukdar, T., & Mukherjee, S. K.(2008). Comparative study of cypselas in three common species of Asteraceae. Pleione, 2(1), 147-149.
29. Tedonkeng Pamo E, Tendonkeng F, Kana J R, Khan Payne V, Boukila B, Lemoufouet J, Miegoue E, Nanda AS (2005): A study of the acaricidal properties of an essential oil extracted from the leaves of Ageratum houstonianum. Veterinary Parasitology 128: 319–323.
30. Tennyson, S., Balaraju, K., Park, K., Ravindran, K. J., Eapen, A., & William, S. J. (2012). In vitro antioxidant activity of Ageratum conyzoides L. (Asteraceae). Asian Pacific Journal of Tropical Disease, 2, 712-714.
31. Tennyson, S., Ravindran, J., Eapen, A., & William, J.(2012). Repellent activity of Ageratum conyzoides L. (Asteraceae) leaf extracts against Anophelesstephensi, Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease, 2(6), 478-480.
32. Tennyson, S., Ravindran, J., Eapen, A., & William, J.(2015). Ovicidal activity of Ageratum conyzoides L. (Asteraceae) leaf extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease, 5(3), 199-203.
33. Veiga, A., Maria da Graça, T. T., Rossa, L. S., Mengarda, M., Stofella, N. C., Oliveira, L. J., ... & Murakami, F. S. (2019). Colorimetric microdilution assay: Validation of a standard method for

determination of MIC, IC50%, and IC90% of antimicrobial compounds. Journal of Microbiological Methods, 162, 50-61.
34. Verma, A., Rizvi, S. M. D., Shaikh, S., Ansari, M. A., Shakil, S., Ghazal, F., & Rehman, A. (2014). Compounds isolated from Ageratum conyzoides inhibit the activity of matrix metalloproteinases (MMP-2 and MMP-9): An oncoinformatics study. Pharmacognosy Magazine, 10(37), 18.
35. Woldeyes, S., Adane, L., Tariku, Y., Muleta, D., & Begashaw, T. (2012). Evaluation of antibacterial activities of compounds isolated from Sida rhombifolia Linn. Malvaceae). Natural Product Chemical Research, 1(101), 2.
36. Zeeshan, M., Rizvi, S. M. D., Khan, M. S., & Kumar, A. (2012). Isolation, partial purification and evaluation of bioactive compounds from leaves of Ageratum conyzoides L. Excli Journal, 11, 78.