PHYTOCHEMICAL SCREENING AND STUDY OF ANTI-MICROBIAL, ANTI-HAEMOLYTIC, ANTI-FUNGAL AND ANTI-OXIDANT ACTIVITIES OF ETHANOL EXTRACT OF *PANICUM SUMATRENSE* LEAVES

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ABSTRACT: Little millets (*Panicum Sumatrense*) are of immense importance both medically and economically. Scientifically proven that the grains of little millets are nutritionally well. In the present research work, the less explored, underutilized, nutrient-rich and easily available *Panicum sumatrense* leaves were analysed for their phytochemical constituents and anti-oxidant or anti-haemolytic activity. The ethanolic extract of the little millet was examined to proximate, phytochemical determinations and anti-oxidant, anti–haemolytic, anti-bacterial and anti-fungal activity. It was found that the given sample contains important Phytochemicals. The extract prepared using leaves of *Panicum sumatrense* was evaluated for their antioxidant activity, anti-haemolytic activity using UV-Vis spectrophotometer. The antibacterial and antifungal activity tests were performed using the Agar well diffusion method. Anti-bacterial activity of ethyl alcohol extract and n-hexane extract was higher for Escherichia coli and In Staphylococcus aureus extract was slightly low effective. Anti-fungal activity of ethanolic and n-hexane extract was not satisfactory. The leaves extract showed that both anti-oxidant, anti-hemolytic and anti-bacterial activity increased with increasing concentration. Thus, the result revealed the anti-oxidant anti-haemolytic and anti-bacterial activity of the underutilized leaves of *Panicum sumatrense.*

Keywords: Little Millet, Chemical analysis, UV-Vis Spectrophotometer, Anti-Haemolytic, Anti-fungal, Agar diffusion method.

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INTRODUCTION

Herbal medicines are always of great importance due to their natural therapeutic potential. Advances in scientific understanding are having a rapid impact on the field of pharmacology. In terms of safety and accessibility, plant-based medicines are ideal (Modaket *at al.,* 2007). Hikmat, Ayurveda, Unani, Siddha, and homoeopathy all recommended using 95% herbs for treatment. (Satyavati *et al.,* 1976). World Health Organization (WHO) estimates that 60% to 80% of total world population relies on plant-based remedies for common ailments (WHO, 2002). A member of the Poaceae family, *Panicum sumatrense* (Little millet) is a common cultivated and medicinal plant. It is widely grown in Africa and Asia's tropical and subtropical regions, even at elevations as high as 7,000 feet above sea level. Drought-resistant little millet in the list of least water-intensive crops in existence.

Panicum sumatrense is the name of the annual grass in question. It has been decided that special forms of this variable species should be developed for cultivation in the drier regions of the tropics, particularly in tropical Asia. Crops such as cereals and grains are grown on plants with dense panicles that droop under the weight of their spikelets as they mature. (Veldkamp *et al.,* 1989; Veldkamp 1996 *et al.,* 1996). A staple food for millions around the world, it is an important source of protein for the indigenous peoples of the region. In addition to being an excellent source of protein, it is also rich in carbohydrates, fats, minerals, and vitamins, making it an essential food (Nirmalakumari *et al.,* 2010).

Table 1. Taxonomical Data of *Panicum sumatrense*

Language	Name
Hindi	Moraiyo, Kutki, Shavan
English	Little millet
Bengali	Sama
Tamil	Samai
Punjabi	Swank

Table 2. The taxonomical data of *Panicum Sumatrance*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Sub phylum	Angiospermae
Class	Monocotyledonae
Order	Poales
Family	Poaceae
Genus	Panicum
Species	*Panicum sumatrense*

MATERIAL AND METHODS

The Study Area: The Quaid-e-Azam Campus, sprawling across 1800 acres of verdant terrain, was masterminded by the celebrated Greek Architect A.C. Doxiadis, the same visionary behind the design of Islamabad. This campus serves as the hub for the university's educational and administrative operations. The presence of a canal, which separates the academic facilities from the student accommodations, enhances the campus's aesthetic appeal**.** Further details are shown in figure 1.

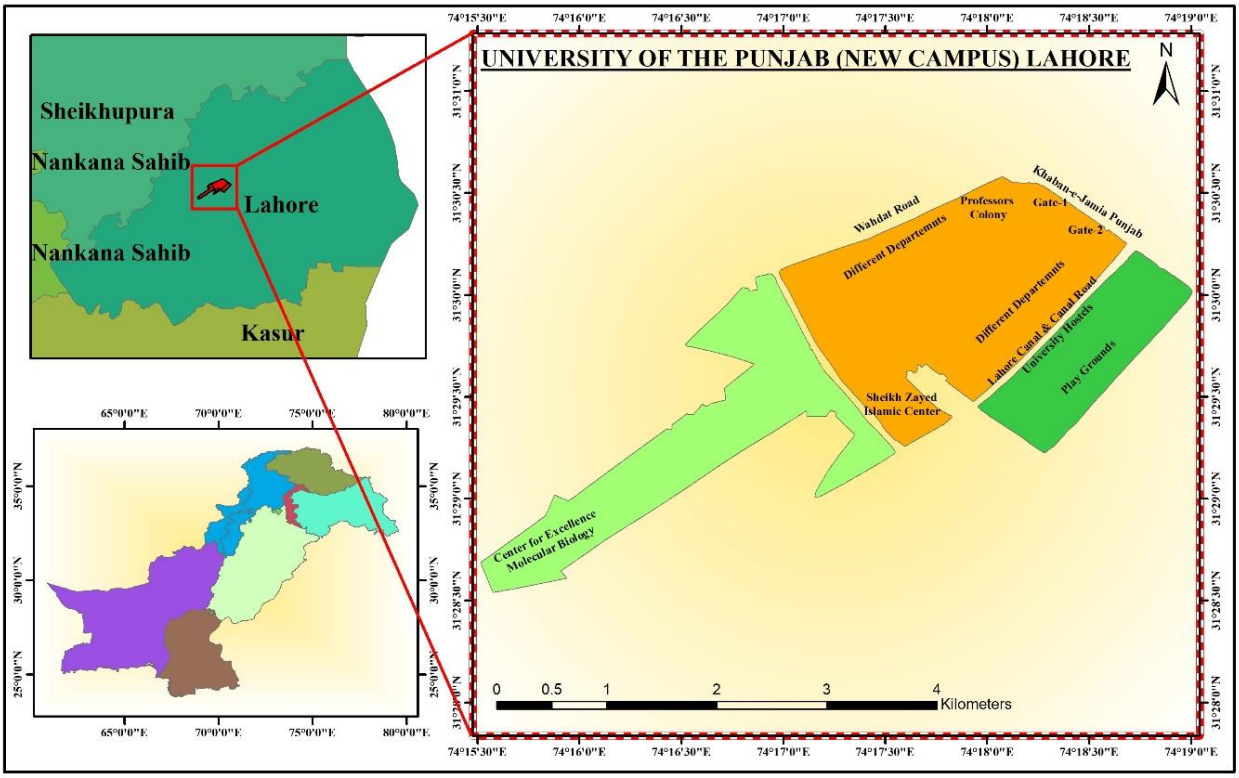

Figure 1. Location Map of the Study Area

Collection of plant material: The *Panicum sumatrense* plant was collected from the Quaid Azam Campus of Punjab University Lahore, Pakistan, and used for this study. After plant collection, their leaves are separated.

Cleaning of Plants: Following the collection of plants, it is necessary to thoroughly clean them. The following steps may be included in the cleaning procedure. Cleaning and washing at least three times are recommended. Cleaning must be done by hand in order to achieve the best possible results.

Drying: Roots are dried primarily for the purpose of removing any remaining water content, which allows the roots to be stored. Roots must be dried as soon as possible after the plants have been collected to avoid spoilage of the plant materials.

Preparation of plant extract: Soxhlet apparatus was used to extract ethanol extract from dried roots of Solanum Xanthocarpum weighing 50 grammes. Using an oven, we were able to remove the ethanol from the extract after the extraction process was complete.

Phytochemical evaluation of plant extract: Small amount of *Panicum sumatrense* ethanolic extract, a qualitative evaluation of phytochemicals in the extract can be carried out (Ranjit *et al.*, 2012).

1. Tannins: When extract mix with the $FeCl_3$ solution it gives dark green color which confirms presence of tannins (Gajare *et al.*, 2012).

2. Flavonoids: When extract mix with NaOH solution it showed yellow color and disappear on addition of dilute acid (Ranjit *et al.*, 2012).

3. Alkaloids:

Hager's test: It was observed for yellow coloration in a small amount of extract when it was mixed with Hager's reagent (Ranjit *et al.,* 2012).

4. *Cardiac Glycosides:* Mix the extract mixture with 5mL of glacial acetic acid containing one drop of $FeCl_3$ solution and 5mL of water with glacial acetic acid. After that, one milliliter of concentrated H_2SO_4 is added, and the formation of a brownish ring indicates the presence of deoxy sugar characteristics in cardenolides (Kumar *et al.,* 2012).

5. Saponins:

Froth test: Saponins are present if the extract is shaken with a few ml of water and the froth remains stable (Kumar *et al.,* 2012).

Phlobatannins: A small amount of the extract was boiled with one percent hydrochloric acid due to the presence of Phlobatannins in it, resulting in the formation of a red precipitate, which was then isolated and further investigated (Kumar *et al.,* 2012).

6. Steroids:

Salkowaski test: Using chloroform and concentrated H2SO4, extract the solution. The chloroform layer has a reddish hue, while the acid layer has a yellowish green (Ranjit *et al.,* 2012)

7. *Terpenoids:* The reddish brown coloration confirms the presence of terpenoids. When a small amount of extract was mixed with 2 millilitres of chloroform, chloroform and H2SO4 were added to the mixture. (Kumar *et al.,* 2012).

8. *Anthraquinone:* Filter extract while still hot after boiling with 10ml dil. H2SO4. Chloroform (5 ml) should be added to the filtrate. Afterwards, shake and transfer the chloroform layer into a test tube containing 1ml of dil. ammonia. Anthraquinone is indicated by a violet color **(**Kumar *et al.,* 2012**).**

Anti-Oxidant Activity (In-Vitro Study): DPPH radical scavenging activity of *Panicum sumatrense* extracts by U.V-Visible spectrophotometry: The DPPH radical scavenging activity of ethanolic extracts of *Panicum sumatrense* was used to determine the antioxidant activity of the extracts.

Principle: At 517 nanometres (nm), 517-nm absorption of the DPPH free radical is the strongest in methanolic solution and is pinkish in colour. Because of the reaction between an antioxidant compound or extract and a stable radical (DPPH), the absorbance of the methanol solution decreases and the colour turns yellow.

$$DPPH + (AH) \text{-} n \longrightarrow DPPH\text{-}H+ (A\text{-}) n$$

(Purple) → (Yellow)

Procedure: An investigation into the radical scavenging activity of extracts against stable DPPH was carried out using spectrophotometry to determine their radical scavenging activity, and the results were published in the journal Antioxidants. It has been observed that the amount of DPPH produced decreases when it interacts with an antioxidant compound that has the ability to donate hydrogen. At 517nm, a UV-Visible Spectrophotometer was used to measure the colour changes (from deep violet to light yellow). At 517 nm, the reduction of the DPPH free radical was used to measure the antioxidant activity.

The newly prepared methanol solution of DPPH of 3ml add in 1ml of the plant extract solution prepared in methanol of different concentration like, 100, 200, 300, 400 and 500ppm. For 30 minutes, the DPPH and plant extract mixture was kept at room temperature in the dark. After that, it used a UV-Visible spectrophotometer to measure the sample's absorbance at 517nm as a control. It was determined using this formula that scavenging activity was extreme:

% of inhibition = (AB – AA / AB) x100

AB Blank absorption

AA Extract Absorption

Anti-Haemolytic Activity (In-Vitro Study)

The following methods are used to prepare human red blood cells (HRBC) suspension: Blood from a healthy volunteer was drawn into a tube containing heparin and stored in a refrigerator. For three minutes, the blood was spun at 1500 rpm in a centrifuge. After collecting the supernatant, plasma was flushed out of the system. Using normal saline, the pallet was washed three times and then centrifuged for five minutes at 1500 rpm. The cells were suspended in normal saline to 2 percent (Gajare *et al.*, 2012) (Kumar *et al.*, 2012)

H_2O_2 Induced Haemolysis: 0.5 mL of a 2 percent HRBC suspension was mixed with 1 mL of extract at various concentrations. The oxidative degradation of red blood cells was induced by incubating the mixture for 5 minutes at room temperature with a 0.3 percent solution of H_2O_2 in normal saline. Extract was omitted from the control. After 240 minutes of incubation at 37 degrees Celsius, the mixture was centrifuged for 10 minutes at 2500 rpm to liberate the haemoglobin and the absorbance at 540 nm was measured to determine the degree of haemolysis. In order to calculate the percentage of haemolysis and the percentage of protection, formulas were used (Prasad *et al.*, 2007).

Haemolysis % = (Absorbance of test sample / Absorbance of control) X 100

Anti- haemolytic activity = Protection % = 100 - (% haemolysis)

Determination of antibacterial activity (in-vitro): The well diffusion method was used to assess antibacterial activity. Freshly prepared bacteria culture was used for this purpose. Bacterial culture of Staphylococcus aureus and Escherichia coli was grown in nutrient broth. Nutrient agar (media) was prepared and after autoclave, bacterial cultures were incorporated into it. Then the media was poured into a Petri dish. After media freezing, 5 mm wells were constructed in each Petri dish using a sterile gel puncture. Four wells were made in each Petri dish. Aqueous leaf extracts of 5 L, n-hexane and *Panicum sumatrense* of each ethanol were then added to the wells of a Petri dish separately at concentrations of 500 / g / ml and 2000 / g / ml. The broad-spectrum antibiotic levofloxacin was used as a control group. It was necessary to keep the plates in an incubator at 37 degrees Fahrenheit for the bacteria to thrive. Measurements were made to determine the size of the inhibitory zone the following day (Perez *et al.,* 1990).

Determination of anti-fungal activity (In-vitro): The fungal culture of Aspergillus niger was grown in potato dextrose agar. The PDA slant was prepared and after these slant sterilization, A. niger was vaccinated. Incubate these PDA slants for three days at 37 ℃. After 3 days, the fungi show growth.

The Agar well diffusion method was used to assess antifungal activity. The Aspergillus niger fungus strain was used. The media, potato dextrose agar, was prepared and vaccinated with fungi after it had been autoclaved. A Petri dish is the best place to put media. After media freezing, 5 mm wells were constructed in each Petri dish using a sterile gel puncture. Four wells were made in each Petri dish. Two concentrations (500 µg / ml and 2000 µg / ml) of leaf extracts were prepared, using ethanol, n-hexane and distilled water. 5 fractions of each were added to the wells of potato dextrose agar (PDA) media. Nystatin (an antifungal drug) was used as a control group. Incubate media for 3 days in 37℃. After three days, the diameter of the inhibitory area was calculated in centimetres and these zone of inhibition shows the effectiveness of the extract (Boyanova *et al.,* 2005).

RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical constituents in medicinal plants make them useful for healing and curing human diseases. (Trombetta *et al.,* 2000). Antioxidants found in medicinal plants, vegetables, and roots are known as phytochemicals because of their ability to protect against a variety of diseases. There are both primary and secondary phytochemicals in plants. Primary constituents include chlorophyll, proteins, and simple sugars, while secondary constituents include terpenoids, alkaloids, and phenolic compounds (Krishnaiah *et al.,* 2007). Anti-inflammatory, anticancer, anti-malarial, cholesterol synthesis inhibition, anti-viral and anti-bacterial activities are just some of the pharmacological properties of terpenoids (Mahato *et al.,* 1997). Terpenoids play a critical role in luring beneficial mites and predatory insects to plants (Kappers *et al.,* 2005). Alkaloids, which are anaesthetics found in plants, are widely used in traditional medicine (Hérouar *et al.,* 1988).

It is shown in Table 3 that the preliminary results of the phytochemical analysis. Various phytocompounds were found in ethanolic extracts, according to the results of the phytochemical study. Tannins, Flavonoids, Saponins, Cardiac Glycosides, Alkaloids, and Terpenoids were absent from Panicum *sumatrense* in the Ethanolic solvent extract except for Phlobatannins and Anthraquinones.

Table 3: **Phytochemical screening of Sorghum halepense leaves.**

Phyto- constituent	Result
Tannins	++
Saponin	++
Flavonoids	++
Cardiac Glycosides	+
Terpenoids	+
Phlobatannins	+
Alkaloids	-
Anthraquinone	-

＋＋ = High amount, ＋ = Small amount, －= Absent

Anti-Haemolytic activity on 2% HRBC suspension: Hydrogen peroxide (H2O2) is found to be very hemolytic on red blood cells. This predicts the oxidative property of H2O2 against cell membrane and liberation of haemoglobin from the cells (James *et al.,* 2014)**.**

In this study we check the leaves extract of *Panicum sumatrense* as an anti-hemolytic drug against H2O2 induced haemolysis on 2% HRBC suspension. The HRBC suspension was treated with H2O2 along with leaves extract of different concentration (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml). A marked reduction in haemolysis was observed. The increase in concentration increases the anti- hemolytic activity and it showed maximum activity at 500µl/ml.

Table 4: **All the values are taken as mean, EEPSL= Ethanolic Extract of *Panicum sumatrense* Leaves.**

Sr. No	Treatment (s)	Concentration µl /ml	Absorbance (A)	Haemolysis (%)	Protection (%)
1	Control DPPH	_-	1.387	_-	-_
2	EEPSL	100	0.155	11.17	88.83
3	EEPSL	200	0.109	7.86	92.14
4	EEPSL	300	0.099	7.13	92.87
5	EEPSL	400	0.081	5.84	94.16
6	EEPSL	500	0.072	5.19	94.81

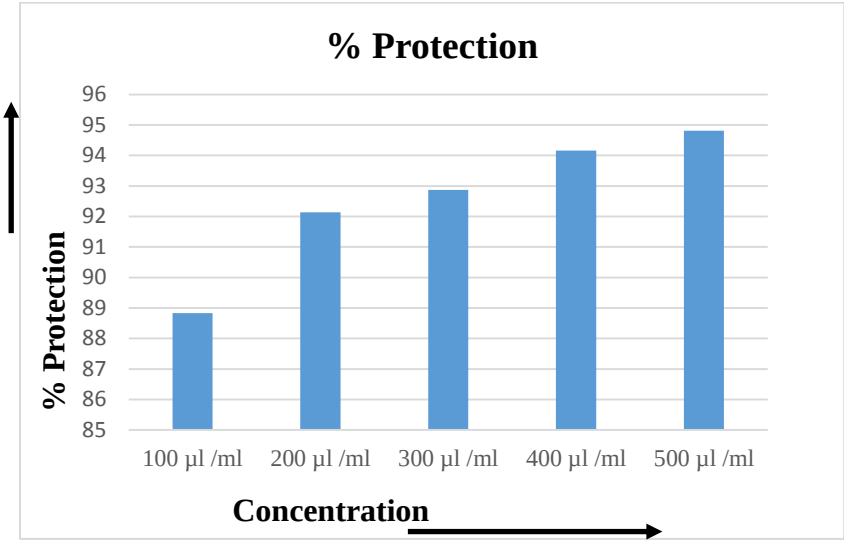

Figure 2. Percentage protection of *Panicum sumatrense* against 2% HRBC suspension

Anti-Oxidant Assay (In-Vitro Study)

Radical scavenging activity DPPH of *Panicum sumatrense* extracts by U.V-Visible spectrophotometry: DPPH radical scavenging was used to measure the antioxidant activity of ethanolic extracts of *Panicum sumatrense*.

Principle: Methanolic DPPH solution, which is pink and has a 517 nm absorption spectrum, is a stable free radical that gives off a 517 nm spectrum. Because of the reaction between an antioxidant compound or extract and a stable radical (DPPH), the absorbance of the methanol solution decreases and the colour turns yellow.

DPPH + (AH) n → DPPH-H + (A-) n
(Purple) ⟶ (Yellow)

Procedure: The extracts were tested for their ability to scavenge radicals using stable DPPH, and the results were recorded using spectrophotometry to determine the effectiveness of their radical scavenging activity. When the antioxidant compound DPPH comes into contact with an antioxidant compound that has the ability to donate hydrogen, the amount of DPPH produced is reduced. At 517nm, a UV-Visible Spectrophotometer was used to measure the colour changes (from deep violet to light yellow). At 517 nm, the reduction of the DPPH free radical was used to measure the antioxidant activity (Williams *et al.*, 1995).

The plant extract solution prepared in methanol of different concentrations like 100, 200, 300, 400, and 500ppm is added to the newly prepared DPPH methanol solution of 3ml. For 30 minutes, the DPPH and plant extract mixture was kept at room temperature in the dark. After that, it used a UV-Visible spectrophotometer to measure the absorbance at 517nm with a black sample as a control. It was determined using this formula that scavenging activity was extreme:

Inhibition % = (AB – AA / AB) x100

AB Blank absorption

AA Extract Absorption

The scavenging effect of different concentration of *Panicum sumatrense* extract (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500µg/ml) on the DPPH free radical was compared with the standard blank solution (without extract). The results are expressed as % inhibition and are depicted in Table. The scavenging of ethanol extract showed considerable activity at all the concentration but the highest activity obtained at a concentration of 500 µg/ ml. This show that greater the concentration of the solution, greater the antioxidant activity.

Table 5: All the values are taken as mean, EEPSL= Ethanolic Extract of *Panicum sumatrense* Leaves.

Sr. No	Treatment (s)	Concentration µg /ml	Absorbance (A)	Oxidation (%)	Protection (%)
1	Control DPPH	-_	2.349	-_	-_
2	EEPSL	100	1.734	73.81	26.19
3	EEPSL	200	1.578	67.17	32.83
4	EEPSL	300	1.538	65.47	34.53
5	EEPSL	400	1.467	62.45	37.55
6	EEPSL	500	0.478	20.34	79.66

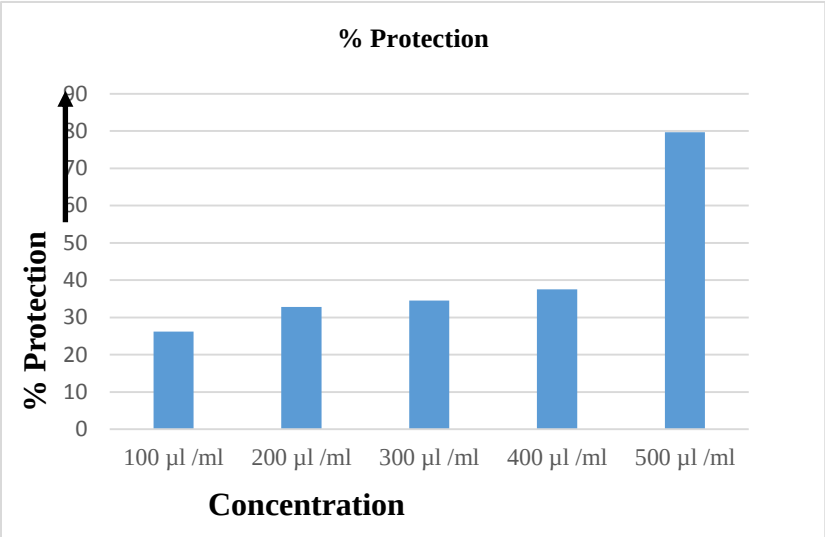

Figure 3. Percentage protection of *Panicum sumatrense* against DPPH radical scavenging activity

Antibacterial activity: Staphylococcus aureus and Escherichia coli were tested for activity against Panicum *sumatrense* leaf ethanol, n-hexane, and aqueous extracts. Commercially available antibiotics Novidat have also been tested on these two distinct bacterial species. A graph and a table were used to display the results. This plant extract from *Panicum sumatrense* showed the greatest inhibition zone against E. Coli bacteria, but no inhibition zone was observed for Staphylococcus aureus. According to the research, alkaloids found in nature and their synthetic derivatives possess a wide range of properties, including analgesic, antisplasmodic, and bactericidal properties (Okwu *et al.*, 2004). It's believed that the compounds in this family, which include rag as well as Cushnie and Oyekunle, exert their antimicrobial effects on bacteria by altering the integrity of their cell membranes (Ragasa *et al.*, 2005, Cushnie and Oyekunle, 2006, Oyekunle and Oyekunle, 2006). (G., Venut *et al.*, 2005). E. coli was inhibited by the given plant extract, but S. aureus was unaffected.

Anti-Fungal activity: Fungal strains of medical importance, such as Aspergillus niger, were tested for activity against Panicum *sumatrense* leaves in ethanol, n-hexane, or aqueous extracts. Nystatin, a commercially available antibiotic, has also been tested on these fungi. No inhibition zone was found in the case of Aspergillus niger. In the case of A. Niger, results showed that the given plant extract had no effect.

Table 6: The values for the zone of bacteria inhibition in water, ethanol and *n*-Hexane.

Sr. No.	Bacteria	Conc. µg/ml	Zone of inhibition in Distilled Water (mm)	Zone of inhibition in Ethanol (mm)	Zone of inhibition in n-Hexane (mm)	Zone of inhibition in Control (mm)
1	**E. coli**	500	0	13	16	38
		2000	0	15	19	
2	**S. aureus**	500	0	0	0	35
		2000	0	0	0	

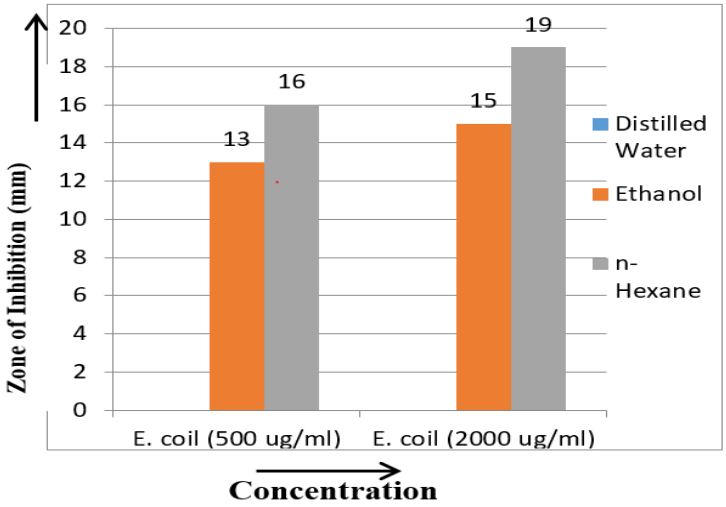

Figure 4. The zone of inhibition of *Panicum sumatrense* leave extract against control group

Conclusion: Phytochemical bioactive compounds, such as tannins, flavonoids, saponins, cardiac glycosides, alkaloids, and terpenoids, are found in medicinal plants, and these compounds are critical in the prevention of a wide range of diseases. Antioxidant, hemolytic, antibacterial and antifungal properties are all attributed to the presence of phytochemicals bioactive compounds in medicinal plants. New drugs can be made with the help of phytochemical constituents discovered and screened in medicinal plants. Drug manufacturers in both academic research institutions and the pharmaceutical industry value phytochemical analyses of medicinal plants when developing new drugs to treat a wide range of ailments. The important phytochemical properties found in Asian plants, as a result of our research, may prove useful in combating diseases specific to this region.

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