Study of Anti-mitotic and Anti-bacterial potential of *Astercantha longifolia* seeds

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Abstract *:*

Since ancient times, plants have been put to use as an alternative and traditionalist medicines to treat a variety of afflictions Natural secondary metabolites are essential in the treatment of a variety of diseases. Phytochemical, antimitotic, and antibacterial attributes of methanolic extract (95 %) of *Asteracantha longifolia* seeds were investigated in this particular study.

Results : To authenticate the existence of phytochemicals such terpenoids, cardiac glycosides, saponins, hormone, phenols, protein, and sugars, phytochemical analysis was carried out utilizing qualitative tests. Meristematic cells from the Allium cepa root were used to test the antimitotic ability. As a reference, colchicine (0.1 mg/mL) was employed as a standard. One-way ANOVA was used to analyze the data using the programmer (GraphPad Prism- 9.5.1) version differences was then identified as those with , < p 0.05 in the statistics was significant. in the case of *A. longifolia* seeds the result obtained was p = < 0.0001 with the antibacterial assay slight zone of inhibition was obtained for *Staphylococcus aureus* and *Bacillus subtilis* of approx.(10 to 15 mm) but no zone of inhibition for *Shigella flexneri* and *Salmonella typhi*

Conclusion : Nearly all phytochemicals are present in significant amounts in *A. longifolia.* It was observed that the plant extract even showed antimitotic activity with having p value less then 0.0001 the antibacterial experiment revealed that plant has slight inhibitory effects for gram positive bacteria and further research can be carried out for the same The lack of antibacterial activity of the plant extract against other gram negative strains was likely due to an error in the selection or implementation of the experimental protocol. Which can further be optimized and evaluate again in future

Keywords: Therapeutic plants , Phytochemical , Anti-mitotic , Antibacterial

Introduction:

Throughout the beginning, people have employed plants as a valuable source of medicine, which laid the groundwork for traditional medicine. These conventional medical plants are essential to meeting today's and the future generations' global healthcare needs [1] The use of medicinal flower extracts for the treatment of any disorder over has seen to be immersed in larger context due to the negative side effects of chemical medications. An abundance of several naturally occurring compounds, including terpenes, flavonoids, phenolics, steroids, and saponins is the main reason for it also Antimitotic, antidiabetic, antidepressant, pain reliever, antipyretic, antiarthritic, antioxidant, antidiuretic, soothing and antibacterial properties are all present in them. Herbs are used as a source of medication for many defined years [2]. Natural products' structural diversity has a significant impact on the development of many contemporary medications [3]. Cancer is the leading cause of mortality among all diseases, and as it is a complex disorder, research into cancer drugs has always held promise. Worldwide efforts are now being made in both developed and developing nations to conduct anticancer agent research. More than half of the anticancer therapeutics derive from natural sources or are linked to them. [4]. The World Health Organization estimates that there were 9.6 million cancer-related deaths globally in 2018 In India, over 7.85 lakh fatalities were reported in 2018 [5]. By 2030, this number could reach 12.0 million. Plants and the materials they produce have been employed in the treatment of cancer for a very long time Exploring traditionally used medicinal herbs is crucial on two levels: first, as a source for potential chemotherapeutic medicines, and second, as a way to gauge their safety for ongoing usage [8]. Based on their use in conventional medicine, the majority of standard anticancer medicines come from natural sources [9].

Unrestrained cell division during mitosis causes cancer. One of the vital factors in the treatment of cancer is the use of antimitotic agents. A prominent class of cytotoxic pharmaceuticals are antimitotic agents, which will persist to be used as a mainstay in cancer chemotherapy in the coming years [10]. Recognizing the bioactivity of plant extracts calls for a knowledge of their phytochemical components. antioxidant substances like By eliminating reactive oxygen species like peroxide and hydroperoxide, phenolic acids, polyphenols, and flavonoids prevent oxidative stress. The antioxidants' ability to prevent DNA damage and unregulated cell division was confirmed by the free radical hypothesis. Flavonoids have a wide range of biological effects, such as antioxidant, antimutagenic, and anticarcinogenic action [11]. Alkaloids from Vinca rosea, Taxus brevifolia originally were used as antimitotic remedy, mainly targeting the localization of tubulin, and posses chemotherapeutic capacity that contributed significantly to the effectiveness of cancer therapy. [12]. It has been demonstrated that the Allium cepa root tip cell is a trustworthy and affordable option for the assessment of antimitotic characteristics [13] Medicinal Plants have antibacterial activity against Pathogens causing Urinary Tract Infections (Sharma et al., 2009). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 1999).]. Extracts of plants contain a variety of bioactive compounds which may inhibit the growth of some pathogenic microorganisms (Zarringhalam et al., 2013). *Asteracantha longifolia,* a medicinal plant that has not received much attention,

has been chosen for this study to assess its antimitotic, antibacterial properties as well as its phytochemical analysis. To check the presence of suitable metabolites *Asteracantha longifolia* (Acanthaceae) is a perennial herb of good medicinal value that is found in large portions throughout India, Sri Lanka, Malaysia, and Nepal. It is widely used as a diuretic and for treating jaundice, kidney stones, rheumatism, liver dysfunction, urinogenital problems, and other conditions [14]

Methods and Materials:

1] Collection and preparation of plant sample

fine powder of seeds of the plant was obtained from ayurvedic store from Rajkot These dried samples pulverized powder form, were employed in further aspects.

2] Preparation of plant extract

Plant material (250 g) was submerged in a 500 ml solvent that was 95% hydro alcoholic (methanol). After 48 hours, solvents were filtered in a beaker using a The Whatman filter paper. Purified yield extracts were produced by drying the filtrate on a rotary evaporator (Odey M.O, et al. 2012). at a rate of 55 °C. development of a dramatically altered method of solvent extraction which is well known. The substance was just dissolved in the solvent after extraction; it wasn't macerated. And kept In a cold, dark location, for further study

3] Phytochemical Testing

A qualitative study was done to determine which significant phytochemical compounds were present in the plant. Several tests were run to check for the validation of alkaloids, and other bioactive compounds such as terpenoids, carbohydrates, amino acids, and so on. Analyzing plants' phytochemical composition Extraction was done following established protocols**.**

1)**Test for flavonoid:-**

Take 2 ml extract, add 10% 2N NaOH. If yellow color observes it means flavonoid is present in extract.

2)**Test for terpenoid:-**

Take 0.5 ml of crude extract. It was treated with 0.2 ml of chloroform and 0.3 ml of concentrated H2SO4. The presence of terpenoids is indicated by the reddish-brown color that appears at the interface.

3)**Test for amino acids:-**

Take 0.5ml of crude extract add few drops of 2% of copper sulphate solution (cuso4). It treats

with 1ml of ethanol followed by excess of potassium hydroxide pellets, if pink color observe it means amino acids are present.

4)**Test for Cardiac glycosidase (Killer Killani Test):-**

Take 0.5 ml extract. Add 0.08 ml glacial acetic acid and 1-2 drops of Fecl3, if brown ring observe & greenish ring may gradually throughout thin layer it indicates the presence of cardiac glycosides.

5)**Test for saponin(Foam test)**

Mix 0.5 ml of extract with small amount of ethanol. 15 minutes lengthwise, it was shaken in a graduated cylinder. If stable foam is observed it means saponin is present.

6)**Test for Steroids (Libermann- Burchared test)**

Take 0.5 ml of crude extract and mixed with 2 ml Acetic Anhydride followed by 2 ml H2SO4.if color was remained add did not change from violet to blue or green it means steroid is present.

7)**Test for Tannins (Ferric chloride test)**

Take 0.5 ml plant extract. Add few drops of 1% Fecl3, if indicate intense green or black color it means presence of tannins.

8)**Test for glycosidase**

Take 2 ml of extract. Add 3ml of chloroform and 10% ammonia solution. If pink color shown it means glycosides is present.

9)**Test for phenol**

Take 2 ml of distilled water then add few drops of 10% ferric chloride in 1ml of the extract. If blue or green color form it shows the presence of phenols.

10) **Test for alkaloid**

Take 1mL plant extract, add 2.5mL of concentrated HCL and mixed in a test tube. Then add few drops of Mayer's reagents. If white precipitate form.it means alkaloid is present.

Antimitotic Assay

From the local market, Allium cepa bulbs (onions) were purchased and set aside for the analysis. Allium cepa root was utilised for the evaluation of antimitotic activity according to a modified approach Fiskesjo (1985) described [24]. Allium cepa bulbs were sown in water and allowed to germinate for 72 hours in a dark, room-temperature environment. The bulbs that formed uniform roots were chosen for additional research (Fig. 1).

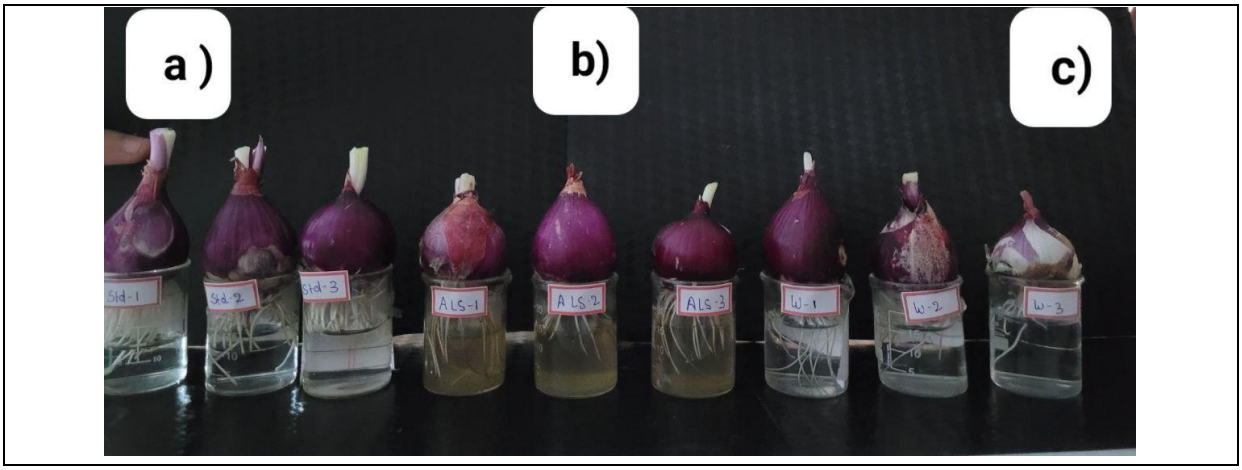


Fig.1 Allium cepa experiment: treatment of root with A- Std , B – Plant Extract , C-Water

During a 24-hour period, onions were placed in a beaker containing water, colchicine (0.1 mg/mL), and plant extracts (10 mg/mL). Colchicine was utilized as a standard for the study and water was used as a control. Samples were run in triplicates. The microscope slide on which the root tips were carefully positioned was covered with a coverslip. To make the cells more visible, they were then dyed with acetocarmine dye. At 10x magnification, the prepared slides were examined using a light microscope. Using CatCam software, dividing and non-dividing cells were counted and visually identified. (Olympus CX21i, Japan) The following formula was used to compute the mitotic index:

$$\text{Mitotic index (MI)} = \frac{\text{dividing no of cells}}{\text{total number of cells}} \times 100$$

Statistical analysis

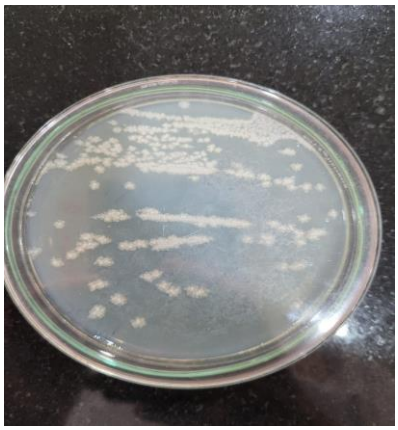
The mean of three replicates and standard deviation were used to express the data by using software GraphPad Prism, Version 6.0 (GraphPad Software Inc., San Diego, The statistically significant difference between controls, standard, and methanolic extracts one-way analysis of variance (ANOVA). $P < 0.05$ is significant value for the presence of the activity

Antibacterial Analysis:

The antibacterial activity of the crude extract was assessed against 2 Gram-positive and 2 Gram-negative bacteria *Bacillus subtilis*, *Staphylococcus aureus* , *Salmonella typhi* , *Shigella flexneri* by producing master plate as positive control for bacteria's fig 2 and using rifampicin antibiotic as a negative control for the same. Fig 3. The organisms were obtained from the Department of m.sc microbiology at virani college in rajkot in pure culture form. The diameter of the zone of inhibition, was measured for the test agents . a loopful of all given bacteria were inoculated into a culture a nutrient broth (1.3%) was prepared for suspension culture. and kept in orbital shaker for 24 hours N-Agar media (40gm/L). was prepared and then poured and spreaded evenly onto petri-dishes then after well diffusion [27] method was followed for the analysis wells were incorporated in total 4 then addition of different concentration of plant extracts was executed and kept in incubator for 24hrs to observe the desire results the concentration were (10mg , 25mg, 50mg, 100mg.) repectively. All medium, broth, Petri plates, tip boxes for micropipettes, and metallic borers were autoclaved at 121 ℃ at 15 psi pressure for 20 minutes to guarantee sterility and avoid contamination.

Shigella flexneri

Salmonella typhi



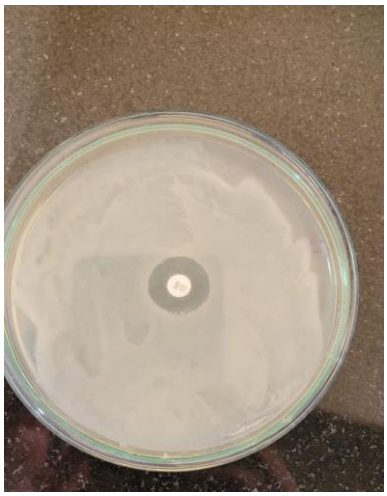
Staphylococcus aureus

Bacillus subtilis

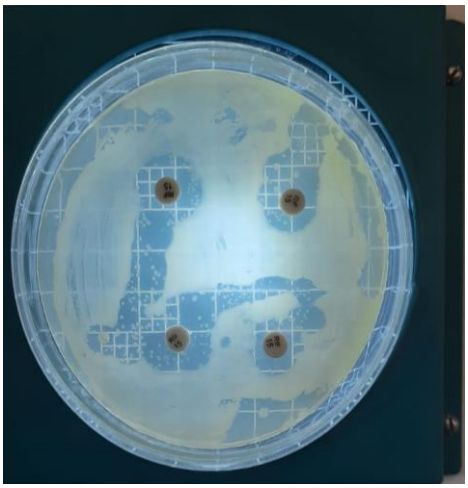
Fig 2 shows the positive control plates

Shigella flexneri

Salmonella typhi



Staphylococcus aureus



Bacillus subtilis

Fig 3 shows the negative control plates

Results:

Phytochemical analysis

According to table1 phytochemical examination revealed that the plant extract included phytoconstituents like terpenoids, saponins, steroids, flavonoids, tannins, cardiac glycosides, amino acids, phenol, and alkaloids.

A. longifolia seed extract contains a large number of these compounds, excluding saponins and amino acids.

Plant extracts' phytochemical profile are listed in Table 1.

Sr.no	PHYTOCHEMICAL ANALYSIS FOR CONSTITUENTS	PLANT EXTRACT
1	Tannins	+++
2	Flavanoids	+++
3	Saponins	-
4	Terpinoids	++
5	Cardic Glycosidic	+
6	Amino acid	-
7	Phenols	++
8	Carbohydrate	+
9	Alkaloids (dragendorff's reg.)	++
	(Mayer's reg)	++
	(Hager's reg.)	+
	(Wagner'sreg.)	++

+ slightly present, ++ moderately present, +++ highly present, − absent,

Antimitotic assay:

Microscopic Observation

The root tip cells was stained with dye known as acetocarmine and then examined under a light microscope. Observed at a magnification of 10X , stained root tip cells can be observed in Fig.4

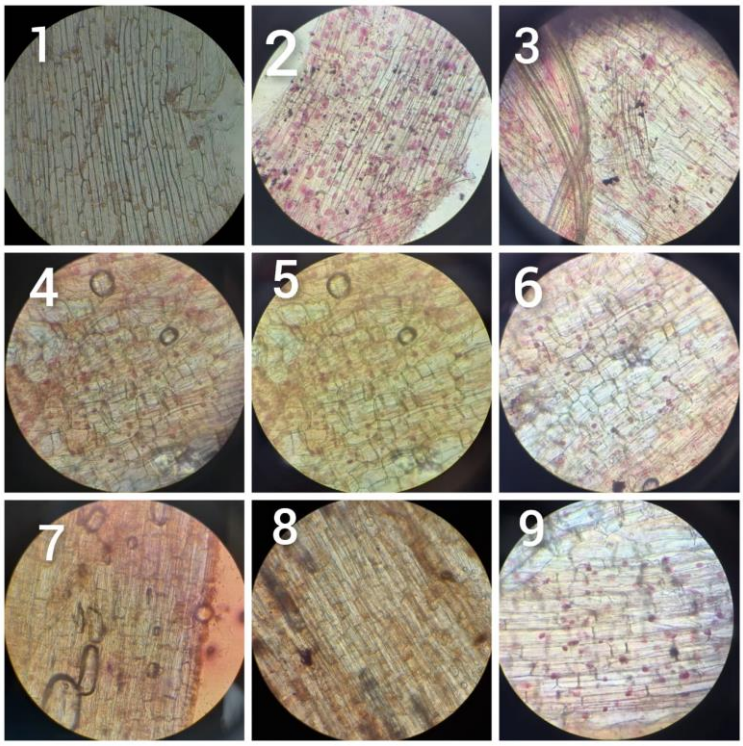


Fig. 4 Microscopic observation at 10× magnification of onion root meristematic cells treated with (123) water, (456) Colchicine (standard) and (789) plant extract: Als

Statistical Analysis:

Software called GraphPad Prism 9.5.1 was used to do the statistical analysis. The graph obtained showed a comparison of the mitotic index of Allium cepa root tips treated with plant, colchicine, and water . Significant antimitotic activity was demonstrated by *A. longifolia* extract, which resulted in a slower rate of mitosis than water. Colchicine, which has the strongest antimitotic activity, was chosen as a benchmark (0.1 mg/mL). $P = < 0.0001$ was derived for *A. longifolia* the significant p value was < 0.05

Table 2 contains the effects of *A. longifolia* on the mitotic index (MI) of Allium cepa root tip cells. As a result, the plants demonstrated considerable antimitotic activity, indicating their potential as a powerful antimitotic agent. (Fig. 5)

Table 2 Effect A. longifolia on mitotic index (MI) of Allium cepa root tip cells

	Water	STD	ALS
Specimen 1	81.27%	29.48 %	41.25%
Specimen 2	75%	24.51%	47.63%
Specimen 3	79%	23.50%	41.25%

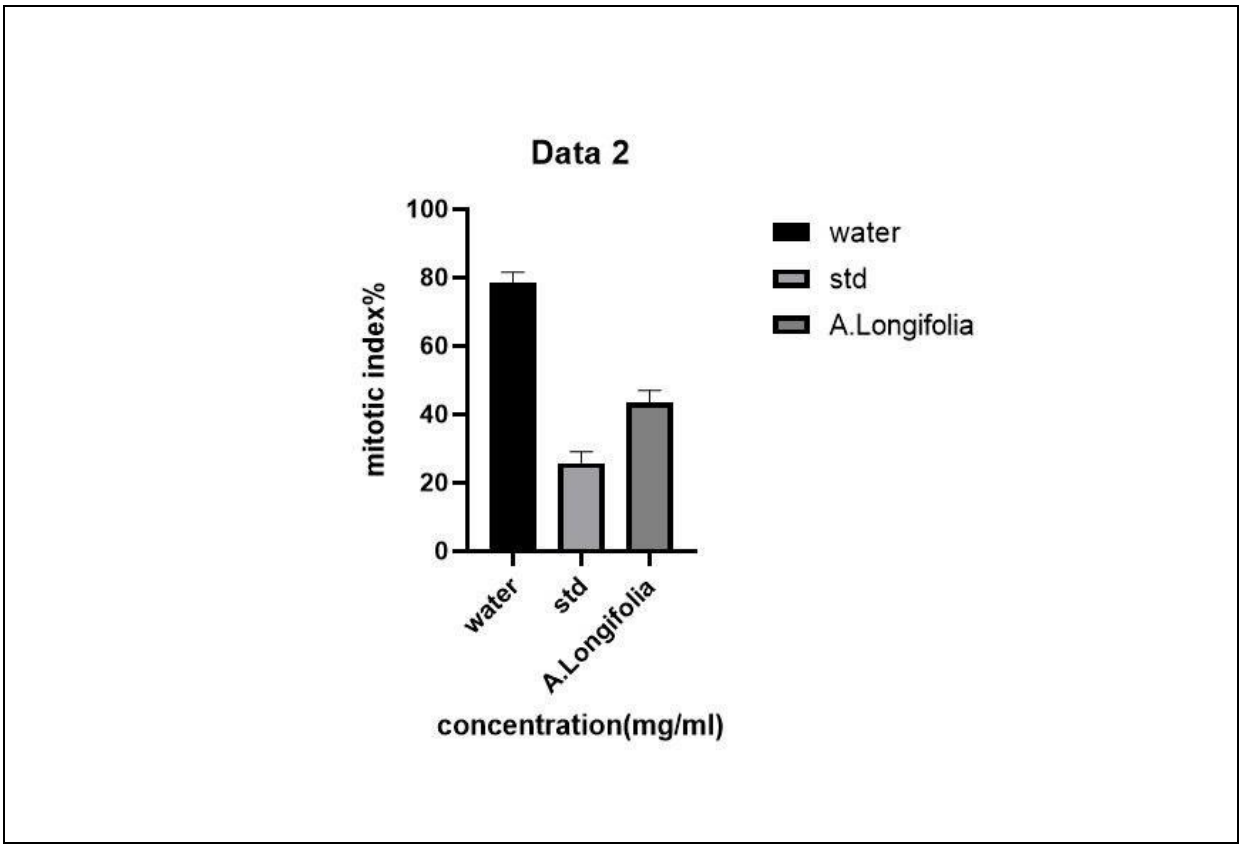


Fig. 5 Compares the mitotic index (MI) of water, std and plant extract.

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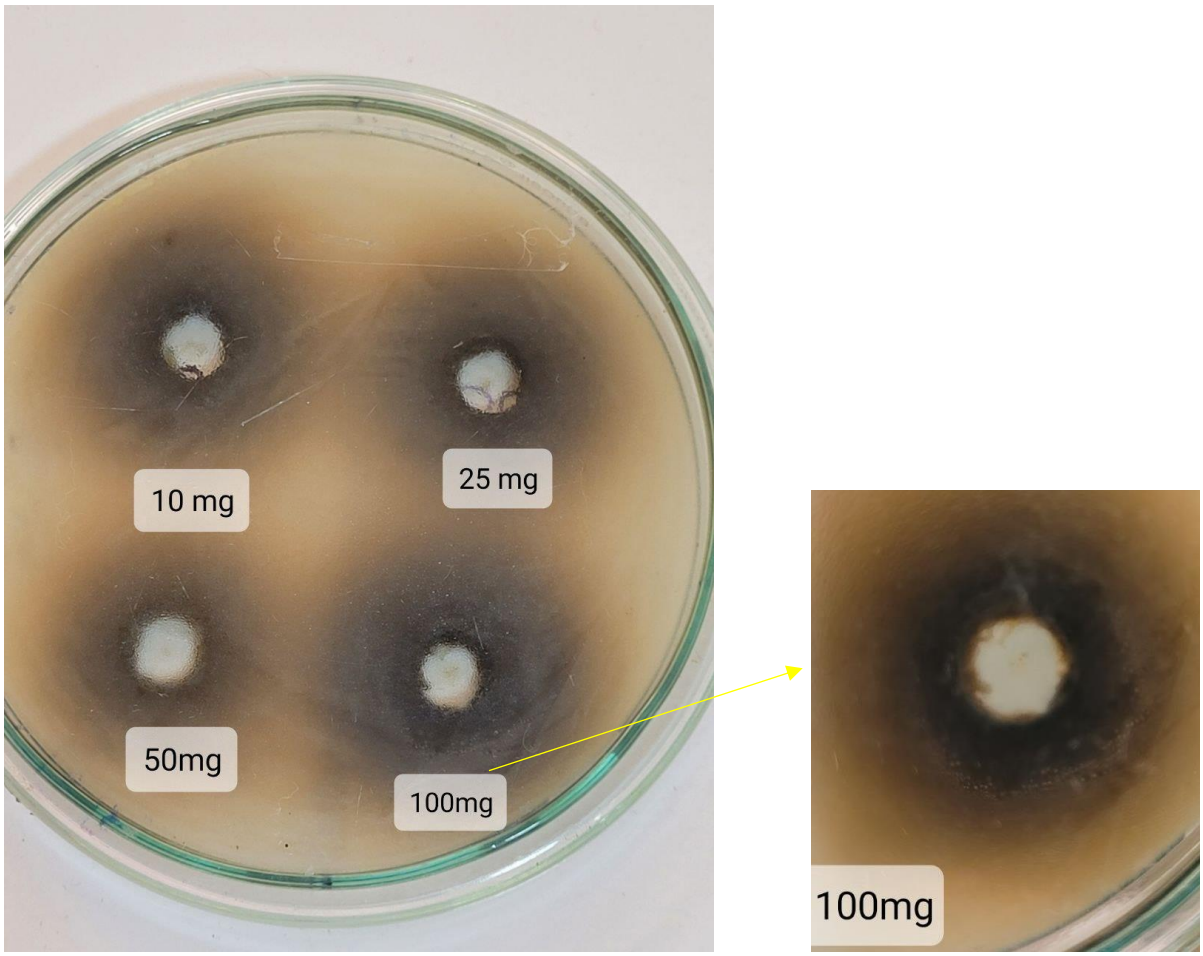
Anti-Bacterial

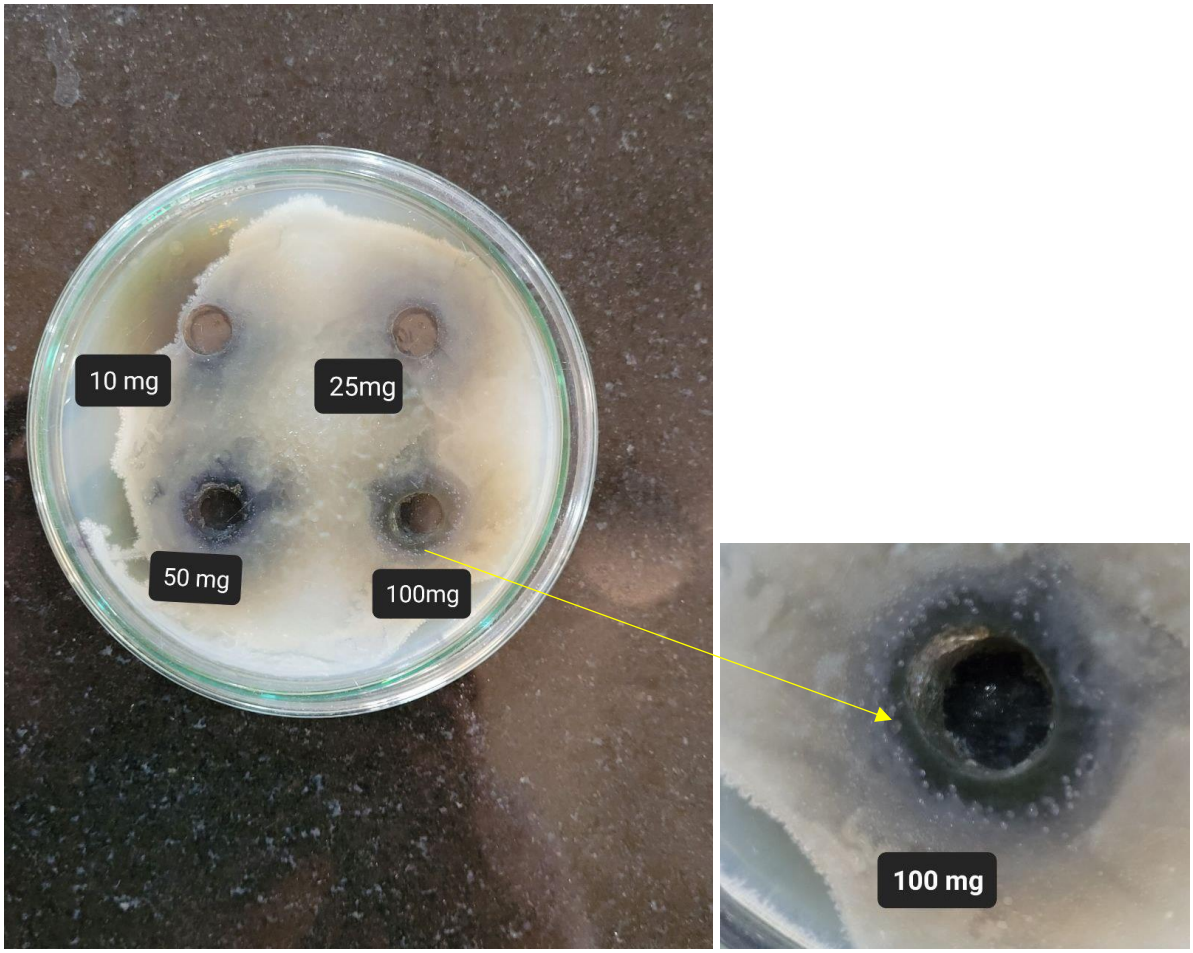
RESULTS:

The performance to check antibacterial properties of the given plant extract by taking its different concentration was determined and zone of inhibition was obtained for two gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (15mm - 10mm) at concentration of 100mg no zone of inhibition were obtained for other gram negative bacteria



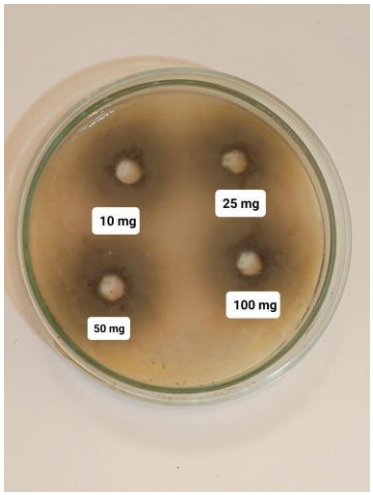
Staphylococcus aureus

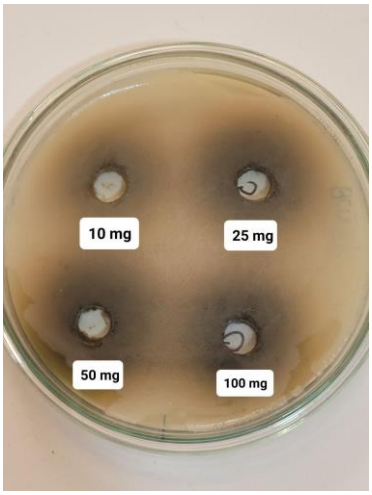
Fig 6 shows antibacterial experiment of plant extract ALS



Bacillus subtilis

Fig 7 Shows antibacterial experiment of plant extract ALS





Shigella flexneri *Salmonella typhi*

Fig 8 Shows antibacterial experiment of plant extract ALS

No zone of inhibition obtained for *Shigella flexneri* *and* *Salmonella typhi*

Conclusion:

The results of the current investigation into the antimitotic and antibacterial characteristics of *A. longifolia* indicate the possibility of such characteristics in the plant. These experiments revealed the presence of significant functionalized substances and biological components in the plant, making it potentially useful for future research in the development of new drugs.

Discussion:

Plants have been used ever since and provide remedies and used in various approaches and have effective properties. there are many studies available on different plants and its parts mentioned in ayurveda by obtaining its crude extract form evaluating its properties and checking its effectivity. In this specific research study was about antimitotic and antibacterial characteristic of the given plant *A.longifolia* the study revealed that the plant posses antimitotic activity and also potential for antibacterial which can be further more explored there are very few papers available for the plant activity which states there should be more potential of different plants of this plant which should be exploited for future studies which can be potent for developing into drug for upcoming diseases as its remedy.

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My sincere thanks to Dr. Praveen S Gupta, an educator at our college who gave us access to cutting-edge tools and workspace. In a time when uncertainty ruled supreme, his constant encouragement was a real lift.

My sincere thanks to Atmiya University for providing me with the opportunity and invaluable advice.

Abbreviations:

ANNOVA= One-way analysis of variance; A. Longifolia = *Astercantha longifolia* MI=Mitotic index

Reference:

1] Sharma S, Kaushik R, Sharma P, Sharma R, Thapa A, Indumathi KP (2016) Antimicrobial activity of herbs against Yersinia enterocolitica and mixed microflora. The Annals of the University Dunarea de Jos of Galati. Food Technol 40(2):119–134

2] Veeresham C (2012) Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res 3(4):200–201. https://doi.org/10.4103/2231- 4040.104709

3] Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol 100(1-2):72–79. https://doi.org/10.1016/j.jep.2005.05.011

4] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin 68(6):394–424. https://doi.org/10.3322/caac.21492

5] Dos Santos HM, Oliveira DF, De Carvalho DA, Pinto JMA, Campos VAC, Mourão ARB, Pessoa C, De Moraes MO, Costa-Lotufo LV (2010) Evaluation of native and exotic Brazilian plants for anticancer activity. J Nat Med 64(2): 231–238. https://doi.org/10.1007/s11418-010-0390-0

6] Richardson MA (2001) Research Conference on Diet, Nutrition and Cancer. Biopharmacologic and herbal therapies for cancer : Research update from NCCAM. J Nutr 131(11):3037–3040. https://doi.org/10.1093/jn/1331.11.3037s

7] Verschaeve L, Kestens V, Taylor JLS, Elgorashi EE, Maes A, Van Puyvelde L, De Kimpe N, Van Staden J (2004) Investigation of the antimutagenic effects of selected South African medicinal plant extracts. Toxicol In Vitro 18(1):29– 35. https://doi.org/10.1016/S0887-2333(03)00131-0

8] Agbafor KN, Nwachukwu N (2011) Phytochemical analysis and antioxidant property of leaf extracts of Vitex doniana and Mucuna pruriens. Biochem Res Int 2011:1–4. https://doi.org/10.1155/2011/459839

9] Kowalczyk E, Krzesiński P, Kura M, Niedworok J, Kowalski J, Błaszczyk J (2006) Pharmacological effects of flavonoids from Scutellaria baicalensis. Prz Lek 63(2):95–96

10] Chauhan NS, Dixit VK (2010) Asteracantha longifolia L. Nees, Acanthaceae: chemistry, traditional, medicinal uses and its pharmacological activities-a review. Rev Bras Farmacogn 20(5):812–817

11] Galani VJ, Patel BG, Rana DG (2010) Sphaeranthus indicus L. A phytopharmacological review. Int J Ayurveda Res. 1(4):247–253. https://doi. org/10.4103/0974-7788.76790

12] de Mesquita ML, Grellier P, Mambu L, de Paula JE, Espindola LS (2007) In vitro antiplasmodial activity of Brazilian Cerrado plants used as traditional remedies. J Ethnopharmacol 110(1):165–170. https://doi.org/10.1016/j.jep. 2006.09.015

13] Brain KR, Turner TD (1975) The practical evaluation of phytopharmaceuticals. Wright-Scientechnica, Bristol

14] Chauhan NS, Dixit VK (2010) Asteracantha longifolia L. Nees, Acanthaceae: chemistry, traditional, medicinal uses and its pharmacological activities-a review. Rev Bras Farmacogn 20(5):812–817

15] Trease GE, Evans WC (2002) Phytochemicals. In: Pharmacognosy. Saunders Publishers, London

16] Mace ME (1963) Histochemical localization of phenols in healthy and diseased banana roots. Physiol Plant 16:915 –925. https://doi.org/10.1111/j. 1399-3054.1963.tb08367.x

17] Wagner H (1993) Pharmazeutische Biology, AUFI. Gustav fisher Vwelag, Stuttgart

18] Fiskesjo G (1985) The Allium test as a standard in environmental monitoring. Hereditas 102(99-1):12

19] Schläger, S., & Dräger, B. (2016). Exploiting plant alkaloids. Current opinion in biotechnology, 37, 155-164.

21] Özmen, A., Basbülbül, G., & Aydin, T. (2007). Antimitotic and antibacterial effects of the Nigella sativa L. Seed. Caryologia, 60(3), 270-272.

22] Evaluation of antimitotic activity of Rotula aquatica (Lour):A traditional herb used in treatment of cancer

23] Çelik, T. A., & Aslantürk, Ö. S. (2006). Anti-mitotic and anti-genotoxic effects of Plantago lanceolata aqueous extract on Allium cepa root tip meristem cells. Biologia, 61(6), 693-697.

24] In vitro antimitotic, antiproliferative and antioxidant activity of stem bark extracts of Ficus benghalensis L

25] Murthy, G. S., Francis, T. P., Singh, C. R., Nagendra, H. G., & Naik, C. (2011). An assay for screening anti-mitotic activity of herbal extracts. Current Science, 1399-1404

27] Başbülbül, G., Özmen, A., Biyik, H. H., & Şen, Ö. (2008). Antimitotic and antibacterial effects of the Primula veris L. flower extracts. Caryologia, 61(1), 88-91.