Pharmacological study of *Anesomeles indica* (leaves)

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Abstract:

Anisomeles indica, a traditional medicinal herb, has been used for centuries to treat various ailments. The objective of the present study was to screen a bioactive compound present in *Anisomeles Indica*. The Sample was prepared by solvent extraction of *Anisomeles indica* using rotary evaporator. Alkaloids, flavonoids, saponins, and tannins were discovered during a phytochemical screening of this plant. In this comprehensive study, various pharmacological assays were performed to evaluate the antimicrobial and anti-mitotic activities of *A. indica* extracts. We performed LC-MS analysis to identify the phytochemical compounds present in the plant. In conclusion, this study demonstrates that *Anisomeles indica* possesses significant antibacterial and anti-mitotic properties, suggesting its potential as a therapeutic agent for bacterial infections and cancer treatment.

Key words

Phytochemical compound, Agar well diffusion method, Anti-bacterial, Anti-mitotic, Anti-cancer

Introduction

Ayurvedic medicine (Ayurveda) is the ancient system of holistic healing and personalized medicine. Developed more than 5,000 years ago in India. Ayurveda's primary objective is to keep the five fundamental constituents in balance (Panchamahabhuta): earth, water, fire, air and vacuum. It is often called the “Mother of All Healing” (Lad, 1984). It comes from the ancient Vedic culture. It is comprised of physical, psychological, philosophical, ethical and spiritual health.

Plants are serving human beings in number of ways; therefore, they are considered gifts from the nature for them. According to WHO 80% of the people in Asia and Africa use herbal medicine for some aspects of primary health care (World Health Organization, 2002). In a

developmental and the under developing country, due to lack of health experts and shortage of medicines, herbal drugs obtained from medicinal plants got more importance (Azmatullah, Suleman, Baqi, Samiullah, & Ayub, 2019). Plants possess a diverse range of bioactive compounds that have the potential to treat various diseases, making them valuable for medicinal purposes. These substances can be extracted from plants and used as tinctures, capsules, or herbal teas, among other ways.

Anisomeles indica, commonly known as "Indian Catmint" or "Kala bhangra" in India, is a medicinal plant that belongs to the Lamiaceae family. This medicinal plant is distributed extensively in Southeast Asia, including Indonesia, Malaysia, Thailand, China, and India. (Daizy, Kaur, Singh, & Kohli, 2007). *Anisomeles indica* has been used in traditional medicine to treat diverse health issues, such as fever, cold, cough, asthma, headache, diarrhea, dysentery, skin diseases, and rheumatic arthritis. (Nasrin, et al., 2022). leaves are commonly used in treating fever and whooping cough in infants. Roots have been used for treating uterine infections, allergies, and mouth infections (Uddin et al., 2018). The extracts obtained during the pre-flowering stage of *Anisomeles indica* it showed anti-histaminergic, free radical scavenging, membrane stabilizing, and cyclooxygenase activities (Dharmasiri et al., 2002). *Anisomeles indica* is known for its rich bioactive compounds, including terpenoids, flavonoids, alkaloids, phenolic acids, and essential oils, which contribute to its medicinal properties (Veena, 2019).

Despite the widespread use of *Anisomeles indica* in traditional medicine, its biological activities have not been extensively studied, and its potential therapeutic applications have not been fully explored. Therefore, there is a need for scientific investigations to evaluate the safety, efficacy, and mode of action of this plant. This dissertation aims to explore the pharmacological and biochemical properties of *Anisomeles indica* and to elucidate its potential as a source of natural medicines in cancer treatments. By conducting a comprehensive review of the literature and experimental studies, this dissertation aims to contribute to the understanding of the medicinal value of *Anisomeles indica* and its potential as a therapeutic agent for various diseases. *anisomeles indica* have antibacterial, anti-mitotic activity, anti-inflammatory activity and also have antidiarrheal activity.

1.Methodology

1.1 collection of sample: -

Crude sample of stem and leaves of *Anisomeles indica* from certified ayruvedic store, dry and grind the sample in powder form.

1.2 Sample preparation:

we performed solvent extraction of *A. indica* using a rotary evaporator (Odey M.O, et al., 2012).

First, 500gm powdered of *A. indica* leaves and stems were mixed with 95% methanol (Nutan & Veena, 2019b). then it was kept in shaker for 48-72hrs. After 48-72hrs mixture was filtered through Whatman filter no 1. The mixture was then placed in a round-bottom flask, which was connected to a rotary evaporator apparatus. The apparatus consisted of a heating bath, a rotating flask, a condenser, and a collection flask. The round-bottom flask was immersed in the heating bath, and the solvent was heated and stirred using a magnetic stirrer.

As the solvent was heated, it vaporized and rose up into the condenser, where it was cooled and condensed back into a liquid. The liquid then flowed into the collection flask, leaving behind the extracted compounds in the round-bottom flask. The rotary evaporation process allowed for the efficient separation of the extracted compounds from the solvent.

Once the solvent had been completely evaporated, the extracted compounds were collected and weighed. The yield of the extraction was calculated based on the weight of the extracted compounds and the weight of the starting material.

1.3 Test for Phytochemical Analysis (Banu, 2015): -

A qualitative phytochemical analysis of *Anisomeles indica* was carried out using the Association of Official Analytical Chemists (AOAC) method. Alkaloids, flavonoids, terpenoids, tannins, saponins, glycosides, phenols, and cardiac glycosides were some of the groups of phytochemicals that were to be identified.

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.

1.4 Antimitotic assay: -

This method was described by Fiskesjo,1985 and Grant,1982 (M.Kavitha2014, *). The activity was assessed using A. cepa root meristematic cells. *Alium Cepa* bulbs were collected, washed, unscaled and grown in 20 ml tap water filled in glass beaker for 95 hours in dark condition. The bulbs that developed uniform roots were selected for the experiment. These Roots were kept for germination under water, cholchicine(1mg/ml) and plant extract(10mg/ml) for 24 hours in dark condition at normal temperature. (Figure 1) Water was used for dilution purposes. Water and Colchicine drug was used as a control. In contrast, plant extract was utilized as the test sample. It performs triplicates the root tips were carefully fixed onto a microscope slide and covered with a coverslip. They were then stained with acetocarmine dye to enhance cell visibility. The prepared slides were observed under a light microscope at both 10x magnifications. Dividing and non-dividing cells were visually identified and counted using CatCam software.

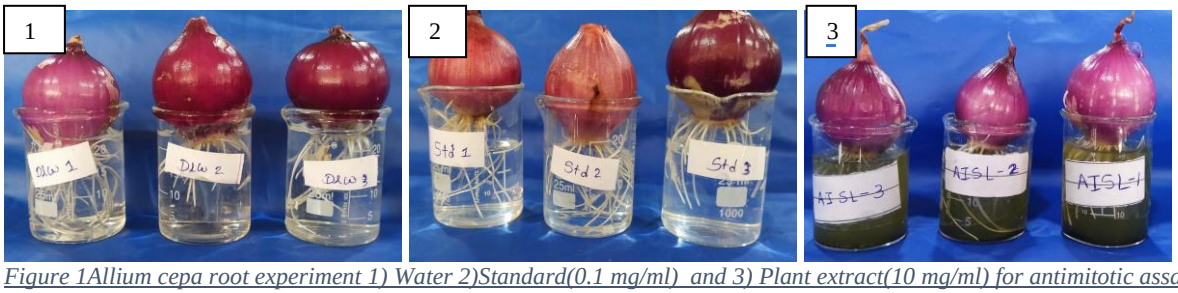

Figure 1Allium cepa root experiment 1) Water 2)Standard(0.1 mg/ml) and 3) Plant extract(10 mg/ml) for antimitotic assay.

To check for the mitotic index, following formula was use:

$$mitotic\ index = \frac{number\ of\ dividing\ cells}{number\ of\ total\ cells} \times 100$$

1.4.1 Statistical analysis: -

Statistical analysis was performed using GraphPad Prism 9 software to determine p values. to analyze significant differences between standard drug, methanolic plant extract, and water by using One-way analysis of variance (ANOVA). if p value less than 0.05 it was considered significant and indicated statistically meaningful result.

1.5 Anti-bacterial activity: -

Media preparation and its sterilization: -

to investigate the antibacterial activity used nutrient agar media(40gm/L). To cultivate the bacterial cells, a Nutrient Broth (1.3%) was prepared for suspension culture. To ensure sterility and prevent contamination, all media, broth, Petri dishes, tip boxes for micropipettes, and metallic borers were autoclaved at 121 ℃ at 15 psi pressure for 20 minutes.

Agar well diffusion method: -

The agar well diffusion method, was initially established by Murray et al. in 1995 and subsequently modified by Olurinola in 1996. The antibacterial activity of plant extract was tested against bacterial strains, *salmonella typhi, Bacillus subtilis, Staphylococcus aureus* and *Shigella*. Master plates of bacterial strains were obtained from the Department of microbiology at *Atmiya University*, Rajkot. A loop full of bacterial colonies from the master plates was transferred to a fresh 100 mL Nutrient Broth for subculture. This was done to initiate a suspension culture and provide the necessary nutrients for the bacterial cells to grow and develop. Then media was poured onto Petri dishes under aseptic conditions. Nutrient agar was providing surface to facilitate colony development of bacteria sterile borers were used to create wells in petri plates. various concentrations of plant extract 10mg/ml,25mg/ml,50mg/ml and 100mg/ml. was added into well using sterile micropipette. The petri dishes were incubated at 37 ˚C for 24 hours. The diameter of the zones of inhibition was measured and compared to the control zone of antibiotics at different concentrations (Guérin-Faublée et al., 1996).

1.2 Results: -

2.1 Phytochemical analysis: -

The qualitative analysis of the phytochemical compounds in methanolic extract of *Anisomeles indica* revealed the presence of flavonoids, alkaloids, steroids, terpenoids, phenols, tannin and cardiac glycoside.

Table 1 Phytochemical profile of methanolic extract of *A.indica*

No.	Phytoconstitute	Test name	*Anisomeles indica*
1	flavanoids	Lead-acetate test	+
2	*Terpenoids*	*Salkowski test*	+
3	*Saponins*	*Foam test*	+
4	*Steroids*	*Libermann-burchared test*	-
5	*Tannins*	*Ferric chloride test*	+
6	*Phenol*	*Ferric chloride test*	+
7	*Carbohydrates*	*Molisch's test*	-
8	*Amino acid*	*Biuret test*	-
9	*Cardic glycoside*	*Killer killani test*	+
10	alkaloids	Dragendroff's test	+

(+) presence of phytoconstituent,

(-) absent of phytoconstituent

2.2Antimitotic assay: -

Microscopic observation: -

Root tip cells were stained with acetocarmine dye and observed under a light microscope at 10x magnification. That result is shown in figure 2.

The impact of the methanolic extract of *Anisomeles indica* on the mitotic index of Allium cepa root cells is given in Table 2.

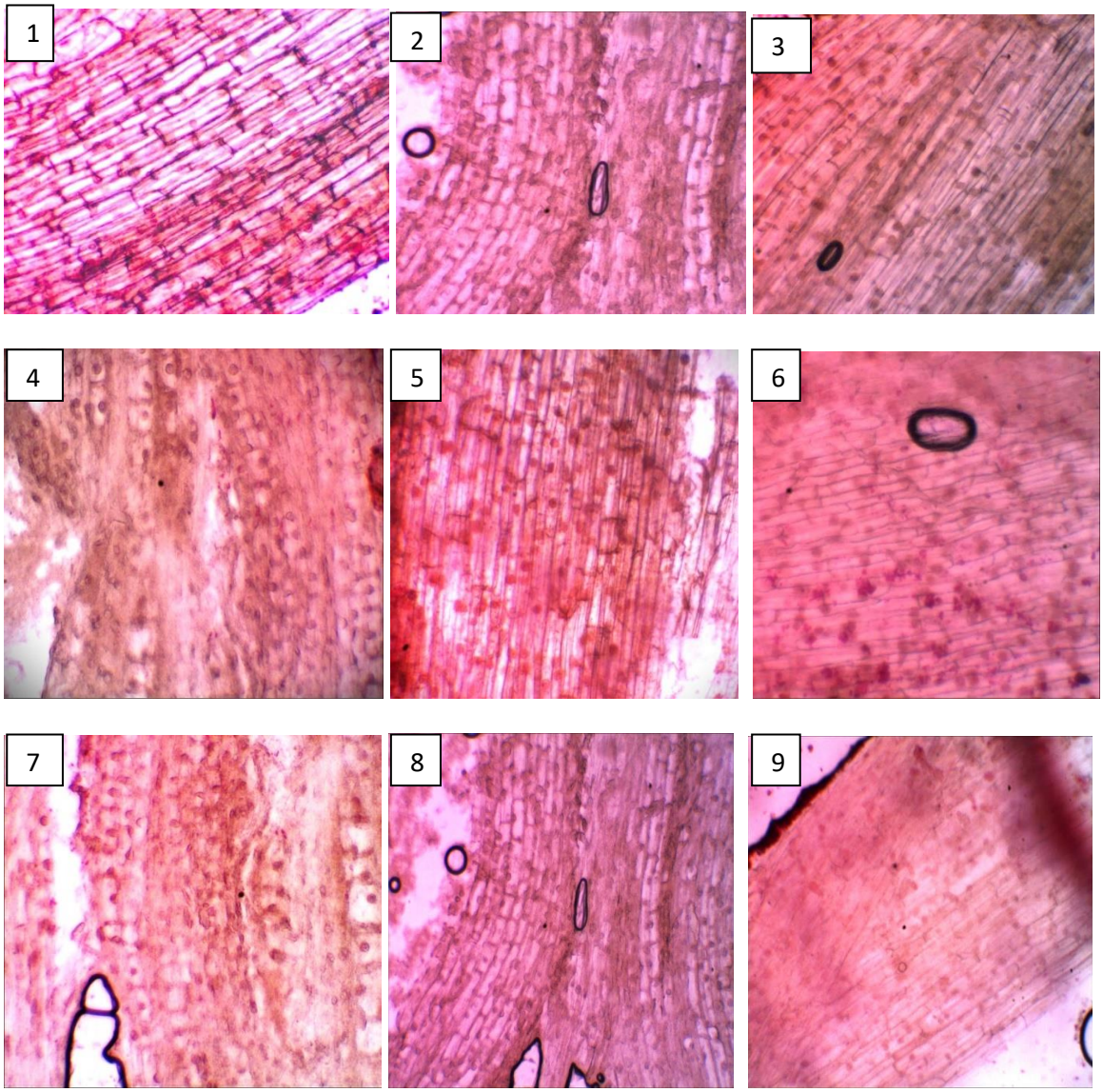

Figure 2 Microscopic observation at 10X magnification, (1-3) water, (4-6) standard, (7-9) methanolic plant extract

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

Treatments	**Mitotic index (%) of *A. indica***
water	82.33
Cholchicine(standard)	15.80
A. indica	60.73

Data represent as mean of three replicates

Statistical analysis: -

Graph pad prism 9 was used for the analysis of statistical data. The mean of triplicates was used to analyses data. The graph displayed a comparison of the mitotic index of Allium cepa roots treated with water, colchicine, and *Anisomeles indica* extracts. Methanolic extract of *Anisomeles indica* showed significant antimitotic activity, by decreasing rate of mitosis in comparison to water. Standard shows highest antimitotic activity's value of ANNOVA analysis is 0. 001.it is less than 0.05 so result is significant. Thus, *Anisomeles indica* displayed significant antimitotic activity which indicates its use as a potent antimitotic agent (figure 3).

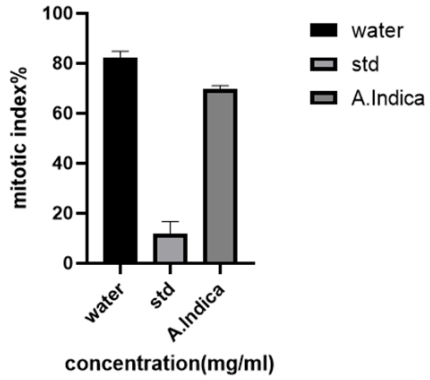

Figure 3 Mitotic index (MI) comparison between water, cholchicine and A.indica extracts

2.3 Antibacterial assay:

In the present study, the inhibitory effect of *Anisomeles indica* was evaluated against different bacterial strains. The antibacterial activity was determined by measuring the zone of inhibition, and the results were compared with the standard antibiotic rifampicin (15µg/ml). Among the various bacterial strains tested, the extract of *Anisomeles indica* showed higher antibacterial activity against Bacillus subtilis, suggest its specific effectiveness against this particular bacterial species. Zone of inhibition diameter is 17mm in which 8mm is the well diameter.it shown in figure 4-5.

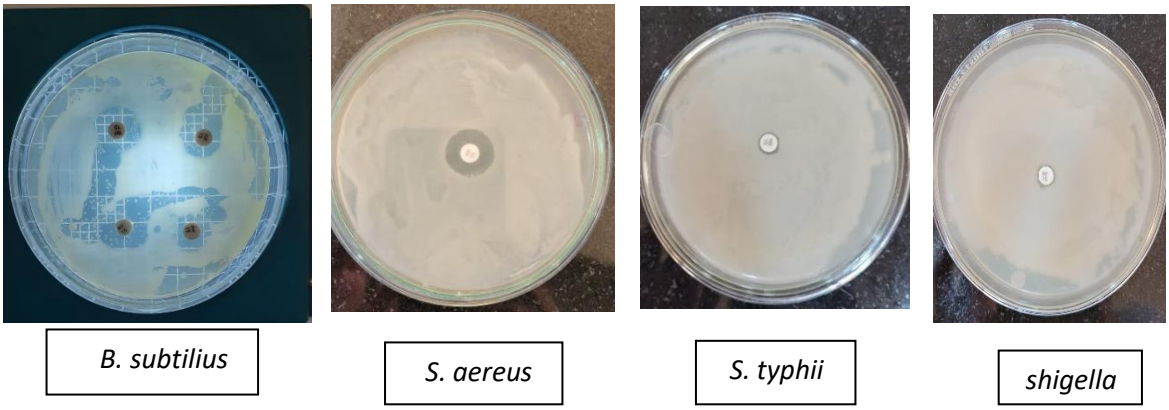

Figure 4 standard drug rifampicin (15µg/ml) effect on different bacterial strains

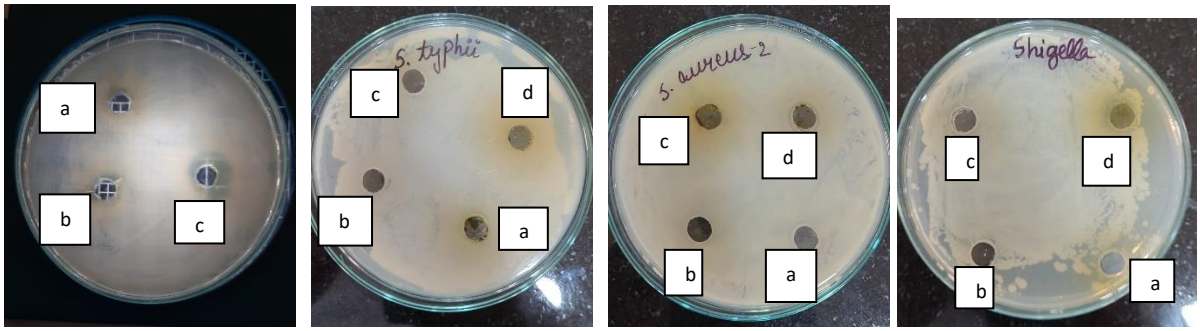

Figure 5 Plant extract effect on different bacterial strains, in each plate (a=10mg/ml) (b=25mg/ml) (c=50mg/ml) (d=100mg/ml)

Discussion: -

Plant derived drug is less toxic and more effective compared to existing drug. Phytoconstituent have significant therapeutic application against pathogens including bacteria, fungi or viruses. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as antimitotic activity.

The present study investigated the potential antibacterial and antimitotic activities of *Anisomeles indica* extract. The results obtained from the experiments demonstrate that the extract exhibits significant activity against bacteria as well as it has antimitotic properties.

Agar well diffusion was used to measure the antibacterial activity of *Anisomeles indica* extract. Antibacterial activity check against pathogenic bacteria, including Gram-positive

(*Staphylococcus aureus and Bacillus subtilis*) and Gram-negative (*Shigella and Salmonella typhii*) strains. extract of *Anisomeles indica* showed higher antibacterial activity against *Bacillus subtilis*, with zones of inhibition ranging from 12 to 17 mm this resulted in its specific effectiveness against *Bacillus substilis*. The lack of antibacterial activity of the plant extract against other bacterial strains was likely due to an error in the selection or implementation of the experimental protocol. Further investigation and optimization of experimental conditions may be required to fully evaluate the antibacterial potential of the plant extract against those specific bacterial strains.

Furthermore, the antimitotic activity of *Anisomeles indica* extract was evaluated using a mitotic index assay on onion root tip cells. The extract significantly decreased the mitotic index in a concentration-dependent manner, indicating its potential to disrupt the normal mitotic process in dividing cells. This suggests that the extract may possess antimitotic properties, which could be utilized for cancer treatment by inhibiting cell division and proliferation.

The observed antibacterial and antimitotic activities of *Anisomeles indica* extract could be attributed to the presence of bioactive compounds, such as alkaloids, flavonoids, and phenolic compounds, which have been use to show antimicrobial and anticancer properties. These bioactive compounds may interact with bacterial cell walls and disrupt their integrity, leading to bacterial cell death. Additionally, the antimitotic activity could be attributed to the ability of the extract to interfere with microtubule dynamics, which are essential for cell division.

Conclusion

The findings of this study highlight the significant antibacterial and antimitotic activities of *Anisomeles indica* extract. These results provide valuable insights into the potential therapeutic applications of this natural extract for the treatment of bacterial infections and cancer. Further studies, including isolation and identification of the active compounds, elucidation of their mechanisms of action, and in vivo evaluations, are warranted to fully understand the therapeutic potential of *Anisomeles indica* extract and its potential use in clinical settings.

Acknowledgments

With the divine grace of Almighty God. I must say, this is the moment when my ship comes in and I am going to submit my research paper on "Pharmacological study of *Anisomeles indica*", At this period of time. I would like to extend my deepest gratitude to our college professor Dr. Praveen Gupta for providing us with state of art materials and facilities for work. His unrelenting support was a genuine boost in a time when uncertainty reigned supreme. No words of thanks can sum up the gratitude that I owe to *Atmiya University* for giving me the opportunity as well as golden guidance. Whist the thesis may only bear my name, I would like to thank all my friends, family, and peers who supported me, and cheered me throughout my research.

Abbreviations

ANNOVA= One-way analysis of variance; *A. indica=Anisomeles indica;* MI=Mitotic index

References

Lad, V. (1984). *Ayurveda: The Science of Self-healing : a Practical Guide*. Lotus Press. https://books.google.co.in/books?hl=en&lr=&id=Tusw0Phg8fMC&oi=fnd&pg=PA13&dq=ayurveda+is+mother+of+healing&ots=eOVQIofDCc&sig=IvHewAyw8J-BBfgrNT00gROlBs&redir_esc=y#v=onepage&q=ayurveda%20is%20mother%20of%20healing&f=false

Muhammad Suleman, A., Samiullah, A., & Ayub, M. (2019). 1. Phytochemicals and their role in curing fatal diseases: A Review. *Pure And Applied Biology (PAB)*, *8*(1), 343-354. Retrieved from https://www.thepab.org/index.php/journal/article/view/746

World Health Organization. (2002). *WHO traditional medicine strategy 2002-2005*. https://apps.who.int/iris/handle/10665/67163

Nasrin, S., Islam, M. T., Tayab, M. A., Nasrin, M. S., Siddique, M. a. B., Emran, T. B., & Reza, A. S. M. A. (2022). Chemical profiles and pharmacological insights of Anisomeles indica Kuntze: An experimental chemico-biological interaction. *Biomedicine & Pharmacotherapy*, *149*, 112842. https://doi.org/10.1016/j.biopha.2022.112842

Uddin, M. N., Asm, A. R., Abdullah-Al-Mamun, M., Msh, K., Nasrin, S., S, A., Msi, A., & Rahman, M. A. (2018). Antinociceptive and Anxiolytic and Sedative Effects of Methanol Extract of Anisomeles indica: An Experimental Assessment in Mice and Computer Aided Models. *Frontiers in Pharmacology*, *9*. https://doi.org/10.3389/fphar.2018.00246

Dharmasiri, M., Ratnasooriya, W., & Thabrew, M. (2002). Anti-inflammatory Activity of Decoctions of Leaves and Stems of Anisomeles indica at Preflowering and Flowering Stages. *Pharmaceutical Biology*, *40*(6), 433–439. https://doi.org/10.1076/phbi.40.6.433.8441

Odey, M. O., Iwara, I. A., Udiba, U. U., Johnson, J. T., Inekwe, U. V., Asenye, M. E., & Victor, O. (2012). Preparation of plant extracts from indigenous medicinal plants. *International Journal of Science and Technology*, *1*(12), 688-692. https://www.researchgate.net/profile/Odey-Oko/publication/259176629_Preparation_of_Plant_Extracts_from_Indigenous_Medicinal_Plants/links/0c96052a200c6c69c1000000/Preparation-of-Plant-Extracts-from-Indigenous-Medicinal-Plants.pdf

Nutan, R., & Veena, S. (2019b). Preliminary phytochemical screening and HPTLC fingerprinting of Anisomeles indica (L.) Kuntze: A traditional medicinal plant. *Journal of Pharmacognosy and Phytochemistry*,*8*(6),25192526. https://www.phytojournal.com/archives/2019/vol8issue6/PartAO/8-5-396-843.pdf

Banu, K. (2015). General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science*, *2*(4), 25–32. https://www.arcjournals.org/pdfs/ijarcs/v2-i4/5.pdf

MKavitha*, P. G. R. a. L. (2019). Evaluation of Antimitotic Activity of Mukia maderaspatana L. Leaf Extract in Allium cepa Root Model. *International Journal of Pharmacy Research and Technology*, *4*(2). https://doi.org/10.31838/ijprt/04.02.01

Oladipupo, A. R., Alaribe, C. S., Akintemi, T. A., & Coker, H. (2021). Effect of Phaulopsis falcisepala (Acanthaceae) Leaves and Stems on Mitotic Arrest and Induction of Chromosomal Changes in Meristematic Cells of Allium Cepa. *Progress in Chemical and Biochemical Research*, *4*(2), 134–147. https://doi.org/10.22034/pcbr.2021.256993.1163

Guérin-Faublée, V., Delignette-Muller, M. L., Vigneulle, M., & Flandrois, J. (1996). Application of a modified disc diffusion technique to antimicrobial susceptibility testing of Vibrio anguillarum and Aeromonas salmonicida clinical isolates. *Veterinary Microbiology*. https://doi.org/10.1016/0378-1135(96)00034-x

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., Yolken, R. H., & Morgan, D. R. (1995). Manual of Clinical Microbiology (6th edn). *Trends in microbiology*, *3*(11), 449-449.

Olurinola, P. F. (1996). A laboratory manual of pharmaceutical microbiology. *Idu, Abuja, Nigeria*, *69*(1996), 1-05.

Sen, A., & Batra, A. (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. *Int J Curr Pharm Res*, *4*(2), 67-73 https://www.researchgate.net/profile/DharmendraArya/post/what_is_the_best_method_to_extract_and_evalute_the_antimicrobien_activity_for_calotropis_procera/attachment/5bbcf9153843b006753d80db/AS%3A679895759851521%401539111189704/download/EVALUATION+OF+ANTIMICROBIAL+ACTIVITY+OF+DIFFERENT+SOLVENT+EXTRACTS+OF+MEDICINAL+PLANT+MELIA+AZEDARACH+L..pdf..