

How to Cite:

Asha, A. K., & Raghavendra, M. P. (2022). Antibacterial activity of isoquinoline isolated from *Andrographis serpyllifolia*. *International Journal of Health Sciences*, 6(S6), 6702–6713. <https://doi.org/10.53730/ijhs.v6nS6.11255>

Antibacterial activity of isoquinoline isolated from *Andrographis serpyllifolia*

Asha A. K.

Postgraduate Department of Microbiology, Maharani's Science College for Women, JLB Road, Mysuru, Karnataka | Research and Development Center, Bharathiar University, Coimbatore | Department of Microbiology. MMK and SDM Mahila Mahavidyalaya. Mysuru. Karnataka

Raghavendra M. P.

Postgraduate Department of Microbiology, Maharani's Science College for Women, JLB Road, Mysuru, Karnataka

*Corresponding author email: ashahsn@gmail.com

Abstract--The medicinal herb *Andrographis serpyllifolia* is used in various ethnobotanical preparations due to its high therapeutic value. In this study, the antibacterial activity of aqueous, hexane, chloroform and methanol extract of *A. serpyllifolia* extracts were tested against human pathogenic bacteria *Campylobacter jejuni*- ATCC 29428, *Neisseria gonorrhoeae*- ATCC 49226, *Haemophilus influenzae*- ATCC 10211, *Enterobacter aerogenes*- ATCC 13048 and *Mycobacterium tuberculosis*- MTCC 300. Among the extracts tested aqueous and methanol extract recorded significant inhibitory activity. Methanol extract was further subjected to bioactivity directed fractionation coupled with isolation and characterization. Purification of the bioactive principle was done by subjecting the crude extract to thin layer, column and HPLC analysis. The fraction exhibiting the antibacterial activity with highest purity and yield was further subjected to ¹H and ¹³C NMR and LC-MS analysis to elucidate its structure. The compound was identified as C₁₇H₂₇NO with IUPAC name 8-(3, 4- dihydroisoquinolin-2(1*H*)-yl) octan-1-ol. Further MIC of the isolated compound against *C. jejuni*, *H. influenza* and *M. tuberculosis* was 500, 500 and 250 µg/ml respectively. The present study is successful in isolating a plant bioactive principle responsible for the antibacterial activity.

Keywords--*Andrographis serpyllifolia*, Antibacterial activity, Isoquinoline compound.

Introduction

Plants are treasure house of medicinally important biological compounds and because of their therapeutic values; they are used as herbal medicine since ancient times (Ali, 2020). Long established practices of traditional medicine in many countries across the world, make use of these medicinal plants. The conventional medicine methods are based on the theories, beliefs, and experiences of different cultures across eras, which are known to maintain, improve the health and also helpful in the prevention, diagnosis and treatment of various diseases (Firenzuoli and Gori, 2007; Castronovo et al., 2021). Among the plant diversity, the family Acanthaceae is mainly consisting of shrubs with 250 genera and 2500 species. The *Andrographis serpyllifolia* of Acanthaceae family is a tall herb found in South India's foothills and damp woodlands. Every part of this plant has been significantly used in the Indian traditional medicine system and Chinese herbal medicine system against fever, cancer, inflammation, snake bites, malaria, digestive disorders, dysentery and diabetes (Damu et al., 1999; Ignacimuthu et al., 2006). *Review of literature on A. serpyllifolia revealed that aqueous and methanolic extract of the plant has antiproliferative and antioxidant properties. The leaves and stem decoctions have been known for their excellent biomedical applications such as anti-bacterial, antidiabetic, anticancer, antiulcer and anti-inflammatory activities. It is a traditionally used herbal medicine against malaria and dysentery in India. The leaf extract is effective against jaundice, cures fever and wounds* (Palithya et al., 2018).

Phytochemical profiles and their possible correlation with antibacterial and antioxidant properties of this plant have been evaluated. Andrographolide, apigenin, 7, 4, 1- dimethyl ether, techtochrysin and serpyllin are reported to be the phytochemical in *A. serpyllifolia* (Venkateshaiah et al., 2009). The anticancer and hypolipidemic activity of *A. serpyllifolia* was proved with respect to its leaf and root extracts by several researchers (Govindachari et al., 1968; Chelluboina and Manga, 2010). The anti-inflammatory property on rat models was also demonstrated by Shankar and Rao (2016). Its inhibitory action of *A. serpyllifolia* against several diseases viz., cancer, diabetes, jaundice, malaria, typhoid etc., was depicted (Rao et al., 2014). The presence of compounds with antimicrobial activity, namely, E-2-Tetradecen-1-01; Pentadecanal; 1, 15-Pentadecanediol; 2-Tetradecanone; 2 – Tetradecanone ($C_{14}H_{28}O$ and Pentadecanal ($C_{15}H_{32}O$), was confirmed via GS/MS chromatogram of *A. serpyllifolia* ethanolic extract (Hemalatha et al., 2017).

Materials and Methods

Collection of plant materials

Aerial parts of A. serpyllifolia were collected from Hassan district, Karnataka. India and identified. The collected plant materials were washed several times with water and shade dried. The stem and leaves of *A. serpyllifolia* were coarse powdered by using waring blender and stored in air tight container for further use.

Plant material extraction

Aqueous extraction

The aerial plant material was macerated in a Waring blender (Waring International, New Hartford, CT, USA) for 10 minutes with 100 mL of sterile distilled water. Before being centrifuged for 30 minutes at 4000 rpm, the macerate was first filtered through two layers of muslin fabric. After sterilization at 120 °C for 30 minutes, the supernatant was filtered using Whatman filter paper No. 1. The extract was stored aseptically at 5 °C until use in a brown container. It was then put through an antibacterial activity test (Raghavendra et al., 2006).

Solvent extraction

25 gms of powdered raw material was dissolved in methanol, chloroform and hexane. Samples were kept in water bath for 4 h for extraction at 50 °C. All the solvent extracts were filtered by using Whatman filter paper No.1 and the extracted solvents were evaporated to dryness using rotary flash evaporator, weighed and preserved for further studies (Harborne 1998; Abubakar and Haque 2020). By using the well diffusion technique, all crude extracts were tested for their ability to inhibit the growth of *Neisseria gonorrhoeae* (ATCC 49226), *Haemophilus influenza* (ATCC 10211), *Campylobacter jejuni* (ATCC 29428), *Enterobacter aerogenes* (ATCC 13048), and *Mycobacterium TB* (MTCC 300).

Antibacterial activity assay

Agar plates containing Soya bean casein digestion were injected with the bacterial solution. Hexane, chloroform and methanol crude extracts were dissolved in methanol. Standard is prepared by dissolving Ciprofloxacin in water which serves as positive control. *E. aerogenes* and *H. influenzae* inoculated plates were incubated chamber for 24 h at 37 °C in aerobic., whereas plates inoculated with *N. gonorrhoeae*, *M. tuberculosis* and *C. jejuni* were incubated in an anaerobic chamber for 24 h at 37 °C. The plates were observed after incubation for inhibition zone if any (Sen and Batra, 2012). Experiments were carried out in triplets and results were recorded as mean of triplicates $\pm$ standard error (ANOVA).

Minimum Inhibitory Concentration (MIC)

Bacterial cultures grown on peptone broth were taken to prepare the cell suspension of 1×10^8 cells / mL. The peptone broth bacterial culture without solvent extract served as control. Different concentrations of plant extracts (3000, 1500, 750, 375, 187, 93.75 and 46.87 μ g/mL) were dissolved in 90 μ L of solvent and were mixed in 96 well plates. Cultures were incubated at 37 °C, broth was observed after 24-48 h and O.D at 590 nm was measured in plate reader. The MIC was determined as the minimum concentration of extract with 50% inhibition as compared with control.

Phytochemical Analysis

The presence of flavonoids, phenols, alkaloids, steroids, saponins, carbohydrates, oils, fats and terpenoids were recorded as described by Harborne (1998). The analysis was made by visual examination of colour change / development of a precipitate following the addition of reagents (Yadav and Agarwala, 2011).

Chromatographic separation of the crude extracts

100 mg of methanol extract was used for Liquid –Liquid (L-L) partition to obtain chloroform, methanol and hexane fractions using separating funnel. TLC plates were prepared using silica gel 0.25 mm with fluorescent indicator F₂₅₄. Sample containing 10 mg / mL of L-L fractions were prepared with respective solvents, 2.5 µL of samples were spotted on TLC plate and allowed to dry. Samples were eluted using chloroform: methanol (9.5; 0.5) mobile phase. Plates were removed after optimal development time then dried and spots were detected at visible light, 366 nm and 254 nm. By using standard formula, R_f values were calculated (Shahverdi, et al., 20017). The HPLC analysis was carried out in Shimadzu LC-Prominence 20 AT using C18 column 250 mm x 4.6 mm, 5µ particle. Samples (10 mg/mL) were prepared from stock respectively in HPLC grade solvents i.e., methanol, chloroform and water. Mobile phase was prepared using mixture of HPLC grade methanol (50%) and 50% HPLC grade water. The flow rate of 1 mL/min was used for separation.

Column fractionation

Sample was prepared by dissolving 1.5 g of MLLCF (Methanol Liquid Liquid Chloroform Fraction; Chloroform fraction obtained from methanol extract) in 4.5 mL of 5% methanol in chloroform. 4 g silica gel H bed was prepared in chromatographic column (300 mm x 15 mm) in chloroform. The prepared sample was loaded to the column and eluted with 120 mL 5% methanol chloroform mixture. 5 mL to 15 mL of fractions were collected in each test tube based on the colour. Fractions with similar pigments were pooled based on the TLC. Column fractions were kept for evaporation. Further, the fractions were analysed by HPLC to check the purity (Bucar et al., 2013).

Spectral analysis

Extracted final compound was subjected to elemental analysis and molecular weight determination as per the standard methods. The structure of the compound was established by ¹HNMR ¹³CNMR and LC-MS (Cos, et al., 2006). To conduct quantitative studies, the ¹HNMR approach was carried out on a 600 MHz NMR spectrometer with optimal acquisition settings. (Yin, et al., 2021. Chemical structure was drawn using ChemDraw.

Results and Discussion

Yield summary of plant material after crude extraction

2.86 g, 71.8 mg and 154.7 mg of dried extracted material were obtained by methanol, hexane and chloroform solvents respectively.

Antibacterial activity of different extracts against test pathogens

All the test pathogens were found to be susceptible against aqueous extracts. Among the pathogens tested *C. jejuni* (18 mm) was found highly susceptible followed by *H. influenzae* (16 mm), *N. gonorrhoeae* (15 mm), *E. aerogenes* (12 mm) and *M. tuberculosis* (12mm). It was observed that the inhibitory activity is not significant compared to positive control against all the test pathogens. Among the different solvent extracts tested, only methanol extract recorded significant inhibitory activity against all the test pathogens. Significant increase in activity was observed compared to aqueous extract. Susceptibility among the test pathogens was also varied. *C. jejuni* (21 mm) was found highly susceptible followed by other pathogens (Table 1).

Minimum inhibitory activity of methanol crude extract

The results confirmed the inhibitory activity of the methanol extract with MIC of 3000, 3000, 1500, 1500 and 750 µg/ml against *E. aerogenes*, *N. gonorrhoeae*, *C. jejuni*, *M. tuberculosis* and *H. influenza* respectively. The hexane and chloroform extracts didn't show inhibitory activity against all the test pathogens (Table 2). 3000, 3000, 1500, 1500 and 750 µg/ml against *E. aerogenes*, *N. gonorrhoeae*, *C. jejuni*, *M. tuberculosis* and *H. influenza* respectively.

Phytochemical analysis

Presence of fats and oils was observed for hexane and chloroform extract along with stain formation whereas it was absent in the methanol extracts. The presence of carbohydrates, alkaloids, terpenoids, steroids, glycosides and phenols in hexane, chloroform and methanol extracts were confirmed. Aminoacids and proteins, oils and fats were present in hexane and chloroform extracts. Saponins and flavonoids tests were positive in methanol extract. Methanol crude extract was selected for Liquid-Liquid extraction as it recorded significant antibacterial activity and MIC against the test pathogens when compared to chloroform and hexane extract.

Yield after L-L Extraction

1.25 gms, 1.32 gms and 20.5 gms of yield were obtained after L-L extraction with methanol, chloroform and hexane respectively from the crude methanol extract.

HPLC Chromatogram

A total of 10, 11 and 13 peaks were observed in methanol, hexane and chloroform extracts respectively; while in MLLMF, a total of 12 peaks were observed, similar number of peaks was noticed in MLLCF. In MLLHF, 13 peaks are observed.

Antibacterial Assay of the Bioactive Principle

Isolated compound exhibited the inhibitory activity against *C. jejuni*, *H. influenzae* and *M. tuberculosis*, whereas, no inhibitory activity was noticed against *E. aerogenes* and *N. gonorrhoeae* (Table 3). The bacterium *M. tuberculosis* showed high susceptibility followed by *H. influenzae* and *C. jejuni*. MIC of the isolated compound was found to be 500, 500 and 250 µg/ml against *C. jejuni*, *H. influenzae* and *M. tuberculosis* respectively (Table 4).

Spectral Analysis

The ^1H -NMR spectra showed signals at δ 1.27 due to methylene groups, the multiple signals at δ 2.03 may be due to methylene group attached to unsaturated system. The bunch of signals at δ 2.88, 3.39 and 3.91 suggest the presence of a glycoside or an alkaloid type molecule. The signals at δ 6.94, 7.36, 7.48 and 7.53 suggest the presence of aromatic protons. In the ^{13}C -NMR spectra of CL-5AF the signals at δ 14.12 is due to methylene group carbon. Along with this the signals at δ 22.70, 25.75, 29.44, 29.63, 29.68, 29.71, 31.94 and 32.81 may be assigned for a number of methylene carbons. The signal at δ 63.11 suggests the presence of a $-\text{CH}_2\text{O}-$ group. The positive mode ESI-MS spectra showed a pseudo molecular ion peak at m/z 401. The elemental analysis data suggest an empirical formula of $\text{C}_{17}\text{H}_{27}\text{NO}$

Proposed structure and details of the isolated compound

^1H NMR (300MHz, CDCl_3) δ = 0.0883 (s, 2H, CH_2), 1.27 (m, 2H, CH_2), 2.86-2.88 (t, 8H, 4 CH_2), 2.89-3.39 (m, 16H, 8 CH_2), 3.50 (t, 2H, CH_2), 3.91-3.96 (m, 12H, 6 CH_2), 7.53-7.28 (4H, ArH) ppm, ^{13}C NMR (300MHz, CDCl_3) = 152.2, 135.5, 130.0, 128.5, 127.3, 127.0, 126.2, 77.3, 77.0, 76.7, 63.1, 32.8, 31.9, 29.7, 29.68, 29.63, 29.3, 25.7, 22.7, 14.1 ppm; m/z = 401(M). Based on the predictions the compound was identified as 8-(3, 4- dihydroisoquinolin-2(1H)-yl) octan-1-ol. The compound was amorphous with melting point 435.26 K. The other parameters observed were critical volume 874.5 $\text{cm}^3/\text{mol.}$, Gibbs energy 237.46 kJ / mol., CMR; 8.1348, exact mass 261.21 and molecular weight; 261.4, C- 78.11, H-10.41, N-5.36, O-6.12.

Discussion

A. serpyllifolia is already reported to possess antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Candida albicans*, *Shigella flexneri*, *Bacillus cereus*, *Shigella boydii*, *Shigella dysenteriae*, *Sternbergia lutea*, *Vibrio parahaemolyticus* and *Vibrio mimicus* (Khurana et al., 2019), *Staphylococcus aureus*, *Streptococcus pneumoniae*,

Pseudomonas fluorescens and *Escherichia coli* (Madhankumar et al., 2020). It was further examined for its inhibitory action against *Campylobacter jejuni* (ATCC 29428), *Neisseria gonorrhoeae* (ATCC 49226), *Haemophilus influenzae* (ATCC 10211), *Enterobacter aerogenes* (ATCC 13048), and *Mycobacterium tuberculosis* (MTCC 300) in light of its broad-spectrum inhibitory activity. In the earlier experiments, these bacteria weren't employed as test organisms. The methanol extract in the current investigation had little inhibitory action against the pathogens that were tested, however hexane and chloroform extracts didn't exhibit any inhibitory activity at all. The previous findings support the present result (Jamuna Bai et.al. 2011).

Hullatti and Rai (2004) stated that after screening *M. malabaricum* leaf extracts in petroleum ether, chloroform, and methanol, only the methanolic extract had antibacterial activity. Their study supports the methanolic extract of *M. malabaricum* leaves' antibacterial activity. There is evidence of broad-spectrum action in the methanolic extract of the leaves against both human and plant diseases. The widespread use of these medications for treating skin conditions is justified by their effective action against *S. aureus*. These may be utilized in the control of plant diseases since the activity was substantial against plant infections as well. In vitro testing of ethanol chloroform, and acetone extracts of *C. religiosum* revealed antibacterial activity, according to Goud et al. (2005). It is obvious that the plant has antibacterial properties when taking into account the earlier research and the most recent findings.

The antibacterial and phytochemical screening of the methanolic extract of *A. serpyllifolia* leaves has not been extensively studied. The methanolic extract of *A. serpyllifolia* leaves, according to Jamuna Bai et al. (2011), had no effect on the other test organisms but had a considerable inhibitory effect on *S. aureus*, *S. typhi* and *X. oryzae* pv *oryzae*. This demonstrates the presence of antibacterial elements in the methanolic extract of the leaves. Venkata S. Kotakadi et.al. 2021 demonstrate a quick and easy approach for creating zinc oxide nanoparticles (ZnONPs) utilizing sodium hydroxide, zinc acetate dehydrate, and a leaf extract of the significant anti-diabetic herb *Andrographis serpelifolia* (As). The findings show that As-ZnONPs have both efficient antibacterial action against gram-positive bacteria and possible antioxidant activity. Additionally, the As-ZnONPs may exhibit in vivo anti-diabetic action (rat model). As-ZnONPs' equivalent antidiabetic effectiveness to that of the conventional medication glibenclamide was shown by a number of biochemical indicators, including HbA1C, serum lipid profiles, and liver and kidney functional markers.

Further phytochemical analysis carried out in the present study is in confirmation with the results published by Palithya et al., (2018). Present study confirms the presence of carbohydrates, alkaloids, terpenoids, steroids, glycosides, phenols in hexane, chloroform, and methanol extracts by the spectral analysis. Amino acids and proteins, oils and fats were present in hexane and chloroform extracts. Saponins and flavonoids tests were positive in methanol extract. Since different phytochemicals had an affinity and solubility in different solvents, the plant extracts showed differences in phytochemical composition. *In general, these constituents of plants are known to have antibacterial, antidiabetic, anticancer, antiulcer, and anti-inflammatory properties.* Additionally, this plant also contains

important chemical constituents such as serpyllin, apigenin 7, 4' – dimethyl ether and tectochrysin which are known for their high medicinal value (Palithya et al., 2018).

The presence of numerous secondary metabolites identified in the earliest phytochemical research may be responsible for these medicinal plants' therapeutic properties. The phytochemical features of the plant extracts may account for the variations in their antibacterial activities. It's likely that some of the plants that weren't effective against specific bacteria still had antibacterial components, but in insufficient amounts to be of any benefit. The findings of the current research are somewhat consistent with how the plants have traditionally been used. Plant-based antimicrobials have tremendous therapeutic promise since they can accomplish the same goals as synthetic antimicrobials while causing fewer negative effects. Plant extracts may also be utilized as biocontrol agents in the treatment of plant diseases since they have shown strong action against plant infections. To identify the antibacterial chemicals found in these plants and to ascertain the range of their activity, further investigation is required. The current investigation of the in vitro antibacterial efficacy of the chosen plants serves as the foundation for further phytochemical and pharmacological investigations.

Conclusion

The present study is successful in reporting the antibacterial activity of 8-(3, 4-dihydroisoquinolin-2(1*H*)-yl) octan-1-ol against five different human pathogenic bacteria having high pathogenicity, mortality, and drug resistance. The structure proposed is based on the spectral analysis and its biological activity was proven with standard procedures. Review of literatures revealed that isolated biomolecule is not reported by earlier workers indicating novelty of the work.

References

1. Abubakar, A.R. and Haque, M., Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of pharmacy & bioallied sciences*, 2020; 12(1), pp.1.
2. Ali, A. Herbs that heal: The philanthropic behavior of nature. *Annals of Phytomedicine*. 2020; 9(1): pp.7-17.
3. Bucar F, Wube A, Schmid M. Natural product isolation-how to get from biological material to pure compounds. *Natural product reports*. 2013;30(4):525-45.
4. Castronovo L.M., Vassallo A., Mengoni A., Miceli E., Bogaani P., Firenzuoli F., Fani R. And Maggini V. Medicinal plants and their bacterial microbiota: A review on antimicrobial compounds production for plant and human health. *Pathogens*. 2021; 10: pp.106-116.
5. Chelluboina, B. and Manga, K., Hypolipidemic activity of *A. serpyllifolia* Wt. Ic. *Pharm Res*, 2010; 3(4), pp.769-770.
6. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of ethnopharmacology*. 2006 19;106(3):290-302.

7. Damu, A.G., Jayaprakasam, B., Gunasekar, D., Blond, A. and Bodo, B. Two acylated flavone glucosides from *Andrographis serpyllifolia*. *Phytochemistry*, 1999; 52(1), pp.147-151.
8. Firenzuoli, Fabio, and Luigi Gori. "Herbal medicine today: clinical and research issues." *Evidence-based complementary and alternative medicine* 2007; 4.S1. 37-40.
9. GOUD PS, MURTHY KS, Pullaia T, BA G. ACTIVITY OF SOME MEDICINAL PLANTS OF NALLAMALAIS, ANDHRA PRADESH, INDIA. *J. Econ. Taxon. Bot.* 2002;26(3).
10. Govindachari, T.R., Parthasarathy, P.C., Pai, B.R. and Kalyanaraman, P.S., Chemical investigation of *Andrographis serpyllifolia*: Isolation and structure of serpyllin, a new flavone. *Tetrahedron*, 1968; 24(24), pp.7027-7031.
11. Harborne, A.J., *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media. 1998;
12. Hemalatha, T., Subha, S. and SaravanaGanthi, A., GC-MS Analysis of the Ethanol Extract of *Andrographis serpyllifolia* (Rottl. ex Vahl.) Wt.(Acanthaceae). *Int J Pharma Res Health Sci*, 2017; 5(6), pp.1923-27.
13. Hullatti, K.K. and Rai, V.R., 2004. Antimicrobial activity of *Memecylon malabaricum* leaves. *Fitoterapia*, 75(3-4), pp.409-411.
14. Ignacimuthu, S., Ayyanar, M. and Sivaraman K, S., Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu (India). *Journal of Ethnobiology and Ethnomedicine*, 2006; 2(1), pp.1-7.
15. Jamuna Bai, A., Rai, V.R. and Pradeepa, V.S., 2011. Evaluation of the antimicrobial activity of three medicinal plants of South India. *Malaysian Journal of Microbiology*, 7(1), pp.14-18.
16. Khurana, S.K., Tiwari, R. and Sharun, K., 2019. Mohd. Iqbal Yattoo, Mudasir Bashir Gugjoo and Kuldeep Dhama, *Embllica officinalis* (Amla) with a Particular Focus on its Antimicrobial potentials: A Review. *J Pure Appl Microbiol*, 13(4), pp.1995-2012.
17. Kotakadi, V.S., Gaddam, S.A., Kotha, P., Allagadda, R., Rao Ch, A. and DVR, S.G., 2022. Bio-inspired multifunctional zinc oxide nanoparticles by leaf extract of *Andrographis serpyllifolia* and their enhanced antioxidant, antimicrobial, and antidiabetic activity—A 3-in-1 system. *Particulate Science and Technology*, 40(4), pp.485-499.
18. Li, J.J., 2014. Pomeranz–Fritz reaction. *Name Reactions: A Collection of Detailed Mechanisms and Synthetic Applications*. Springer, pp.490-491.
19. Madhankumar, R., Sivasankar, P., Kalaimurugan, D. and Murugesan, S., Antibacterial and Larvicidal Activity of Silver Nanoparticles Synthesized by the Leaf Extract of *Andrographis serpyllifolia* Wight. *Journal of Cluster Science*, 2020; 31(4), pp.719-726.
20. Palithya, S., Kotakadi, V.S., Pechalaneni, J. and Challagundla, V.N., Biofabrication of silver nanoparticles by leaf extract of *Andrographis serpyllifolia* and their antimicrobial and antioxidant activity. *International Journal of Nano Dimension*, 2018; 9(4), pp.398-407.
21. Palithya, S., Kotakadi, V.S., Pechalaneni, J. and Challagundla, V.N., Biofabrication of silver nanoparticles by leaf extract of *Andrographis serpyllifolia* and their antimicrobial and antioxidant activity. *International Journal of Nano Dimension*, 2018; 9(4), pp.398-407.

22. Raghavendra, M.P., Satish, S. and Raveesha, K.A., In vitro evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica*. *Journal of Agricultural Technology*, 2006; 2(1), pp.77-88.
23. Rao, C.H.V., Azmi, L. and Gupta, S.S., Phytochemical and pharmacological review on *Andrographis serpyllifolia*: Potential herbal cure-all. *Mintage Journal of Pharmaceutical Medical Science* 2014; 3(3), pp.1-3.
24. Sen, A. and Batra, A., Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azadirach* L. *Int J Curr Pharm Res*, 2012; 4(2), pp.67-73.
25. Shahverdi AR, Abdolpour F, Monsef-Esfahani HR, Farsam H. A TLC bioautographic assay for the detection of nitrofurantoin resistance reversal compound. *Journal of Chromatography B*. 2007 May 1;850(1-2):528-30
26. Shankar, R. and Rao, B.G., Anti inflammatory Activity of *Andrographis Serpyllifolia* Root Extract in Experimental Animals. *Biosciences Biotechnology Research Asia*, 2016; 5(1), pp.483-485.
27. Suryasa, I. W., Rodríguez-Gómez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, 5(2), vi-ix. <https://doi.org/10.53730/ijhs.v5n2.2937>
28. Venkateshaiah, S.U., Jayaram, S. and Dharmesh, S.M., Antiproliferative, antioxidant and cyto/DNA protective properties in *Andrographis serpyllifolia*: role of andrographolide and phenolic acids. *Journal of Complementary and Integrative Medicine*, 2009; pp. 6(1).
29. Widyaningrum, I. ., Wibisono, N. ., & Kusumawati, A. H. . (2020). Effect of extraction method on antimicrobial activity against staphylococcus aureus of tapak liman (*elephantopus scaber* l.) leaves. *International Journal of Health & Medical Sciences*, 3(1), 105-110. <https://doi.org/10.31295/ijhms.v3n1.181>
30. Yadav, R.N.S. and Agarwala, M., Phytochemical analysis of some medicinal plants. *Journal of phytology*, 2011; 3(12). pp.10-14.
31. Yin, T., Lu, J., Liu, Q., Zhu, G., Zhang, W. and Jiang, Z., 2021. Validated Quantitative ¹H NMR Method for Simultaneous Quantification of Indole Alkaloids in *Uncaria rhynchophylla*. *ACS omega*, 6(47), pp.31810-31817.

Table 1
Antibacterial activity of different extracts against test organisms

Test organisms	Antibiotic/Extracts	Conc. per well (µg/ml)	Zone of inhibition (mm)
<i>C. jejuni</i>	Control	-	-
	Ciprofloxacin (Standard)	2.5	30 ±0.577
	Aqueous extract	2500	18± 0.290
	Hexane	2500	-
	Methanol	2500	21± 0.208
	Chloroform	2500	-
	Control	-	-
<i>N. gonorrhoeae</i>	Ciprofloxacin (Standard)	2.5	23±0.577
	Aqueous extract	2500	15±0.312
	Hexane	2500	-

<i>H. influenzae</i>	Methanol	2500	18±0.301
	Chloroform	2500	-
	Control	-	-
	Ciprofloxacin (Standard)	2.5	24±0.577
	Aqueous extract	2500	16±0.280
	Hexane	2500	-
	Methanol	2500	20±0.208
	Chloroform	2500	-
	Control	-	-
	Ciprofloxacin (Standard)	2.5	25±0.577
<i>E. aerogenes</i>	Aqueous extract	2500	12±0.290
	Hexane	2500	-
	Methanol	2500	15±0.290
	Chloroform	2500	-
	Control	-	-
	Ciprofloxacin (Standard)	2.5	28±0.577
<i>M. tuberculosis</i>	Aqueous extract	2500	12±0.310
	Hexane	2500	-
	Methanol	2500	20±0.312
	Chloroform	2500	-

Values are mean of triplicates ± Standard Error

Table 2
Minimum inhibitory activity of methanol extract

Sample Conc. (µg/ml)	Methanol extract <i>C. jejuni</i>		<i>H. influenzae</i>		<i>E. aerogenes</i>		<i>M. tuberculosis</i>		<i>N. gonorrhoeae</i>	
	OD	% Inhibition	OD	% Inhibition	OD	% Inhibition	OD	% Inhibition	OD	% Inhibition
0	0.568	0.00	0.559	0.00	0.586	0.00	0.569	0.00	0.569	0.00
46.87	0.508	10.57	0.498	10.99	0.533	9.02	0.504	11.31	0.520	8.73
93.75	0.437	23.05	0.439	21.47	0.479	18.15	0.458	19.38	0.460	19.16
187.5	0.390	31.30	0.369	33.98	0.437	25.31	0.396	30.27	0.420	26.17
375	0.338	40.51	0.318	43.14	0.386	34.10	0.350	38.47	0.387	32.05
750	0.298	47.51	0.244	56.34	0.349	40.40	0.291	48.90	0.348	38.92
1500	0.248	56.32	0.197	64.81	0.302	48.48	0.242	57.52	0.309	45.80
3000	0.179	68.46	0.158	71.75	0.257	56.08	0.198	65.26	0.276	51.55
MIC	1500µg/ml		750µg/ml		3000µg/ml		1500µg/ml		3000µg/ml	

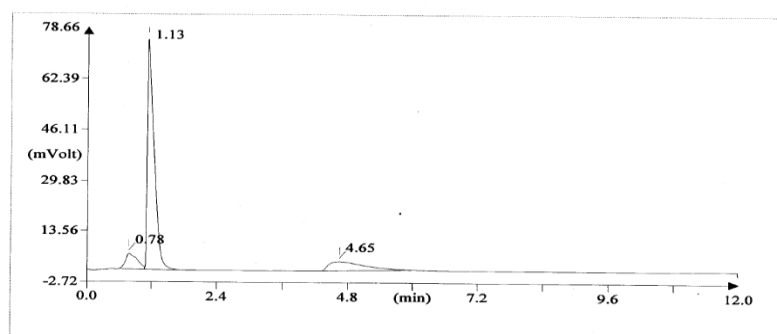
Table 3
Antibacterial activity of isolated compound

Test (Conc. per well 1.0 mg)	Compounds	Zone of inhibition (mm)				
		<i>C. jejuni</i>	<i>N. Gonorrhoeae</i>	<i>H. influenza</i>	<i>Ent. aerogenes</i>	<i>M. Tuberculosis</i>
Control(Methanol)		-	-	-	-	-
Ciprofloxacin (Standard) (S)		15±0.577	20±0.577	18±0.577	28±0.577	17±0.577

Isolated Compound	12±0.318	-	14±0.208	-	16±0.318
Values are mean of triplicates ± Standard Error					

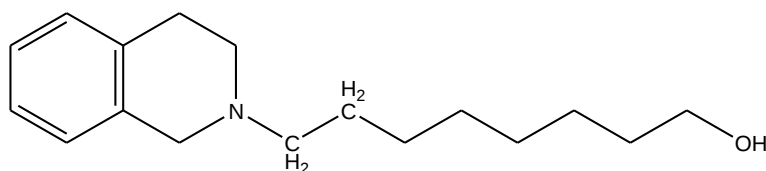
Table 4
Minimum inhibitory activity of isolated compound

Isolated compound	MIC (µg/mL)				
	<i>C. jejuni</i>	<i>H. influenzae</i>	<i>E. aerogenes</i>	<i>M. tuberculosis</i>	<i>N. gonorrhoeae</i>
Standard (Ciprofloxacin)	1	0.5	1	0.5	0.5
Isolated compound	500	500	NA	250	NA



Element Name	Element %	Ret. Time
Nitrogen	3.78	0.78
Carbon	40.32	1.13
Hydrogen	5.56	4.65

Fig. 1. LC-MS profile chromatography for the eluted purified compound



8-(3,4-dihydroisoquinolin-2(1H)-yl)octan-1-ol

Fig. 2. Structure of the bioactive principle