Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [2] 2023: 495-500.

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial Activity of Compounds Extracted from *Sida rhombifolia* Linn.

Ankit Kumar Saini[1], Arun Nivas Velumani[2], Hari V.[3*], Suhas Narayan Sakarkar[4], Brijesh Kumar Singh[5], Mohammad Rizwan[6], Abhishek Amod Gupta[7*]

[1] Krishna Pharmacy College, Bijnor, Uttar Pradesh, India
[2] Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, 600106, India
[3]Department of Pharmacology, MB School of Pharmaceutical Sciences (Erstwhile Sree Vidyaniketan College of Pharmacy), Mohan Babu University, Tirupati, Andhra Pradesh, 517102, India
[4]School of Pharmacy, G. H. Raisoni University, Saikheda, Chhindwara, 480337, Madhya Pradesh, India
[5]Department of Pharmacy, Sri Paramhansh Shikshan Prashikshan Mahavidyalaya, Vidyakund Ayodhya Dist-Ayodhya, Uttar Pradesh, India
[6]Assistant Professor, Department of Pharmaceutical Sciences, Kumaun University, Bhimtal, Nainital, Uttarakhand, India
[7]Assistant Professor, Department of Pharmacology, Government Pharmacy Institute, Agamkuan, Patna, Bihar University of Health Sciences, Patna, Bihar, 800007, India

ABSTRACT

The main aim of this investigation was to extract chemicals from the roots of Sida rhombifolia and afterwards assess their antibacterial properties. Crude gradient extracts were acquired through the utilization of the cold maceration technique from three solvents, namely petroleum ether, chloroform, and methanol, which were selected based on their increasing solvent polarity. The present study aimed to assess the antibacterial efficacy of gradient extracts and extracted chemicals by in vitro experimentation. Four harmful bacterial strains, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhimurium, were selected for examination. The agar disc diffusion technique was employed as the method of analysis. The findings indicated that the antibacterial activities exhibited similar levels of effectiveness. However, the actions exhibited by the tested compounds were comparatively less potent when compared to the reference agent, ciprofloxacin. Out of the three crude extracts, the chloroform extract was utilized for column chromatographic separation, resulting in the isolation of SRL-1, SRL-2, and SRL-3. The antibacterial activity identified in the crude extracts and isolated components provide support for the traditional medicinal application of the plant in treating various bacterial illnesses. Therefore, it is advisable to conduct additional testing on a substantial number of bacterial strains in order to determine the potential of the compounds as candidates for the creation of antibacterial medications.

Key words: *Extraction, isolation, and antibacterial activity of Sida rhombifolia; Stigmasterol, n-Hexacos-11-enoic acid, and –Sitosterol*

Received 30.09.2023 Revised 25.10.2023 Accepted 31.11. 2023

INTRODUCTION

The utilization of natural resources for the treatment of a diverse range of ailments in both humans and domestic animals has a longstanding historical association. These products have been utilized as valuable resources for the development of numerous contemporary pharmaceuticals aimed at treating various human ailments including cardiovascular diseases, cancer, malaria, mental disorders, and others [1, 2]. The majority of contemporary pharmaceuticals have been derived or identified from botanical sources with therapeutic properties. These pharmaceutical substances have been identified through the examination of the therapeutic applications of a certain botanical species or its components by practitioners of herbal medicine. Subsequently, bioactive chemicals have been extracted from the plant or its specific sections that have traditionally been employed in the treatment of various human ailments. The aforementioned data collectively suggest that medicinal plants possess a significant potential as viable resource for the development of contemporary pharmaceuticals. *Sida rhombifolia* is classified as one of the 200 species within the genus Sida [3-5].

The plant exhibits growth patterns characteristic of tropical and warm environments, and its distribution spans throughout many tropical areas. *Sida rhombifolia* Linn is renowned for its extensive array of therapeutic applications [6]. For example, this substance is utilized in the treatment of scorpion, snake, and wasp stings and bites, as well as various skin diseases and sores. It is also employed to address stomach disorders, including stomach pain, digestion issues, and conditions such as malaria, flatulence, diarrhea, dysentery, irritable bowel syndrome, gastritis, enteritis, and hemorrhoids [7]. Additionally, it is used in the management of diabetes, chickenpox, blood purification, fatigue, headaches, including migraines, eye problems such as conjunctivitis, toothaches, fever, gum infections, swelling, and as a tonic for general well-being. Lastly, it is applied topically to wounds, ophthalmic conditions, cuts, and to reduce swelling. *Sida rhombifolia* is extensively utilized by herbalists in Ethiopia for the treatment of many human ailments. Scientific investigations have been conducted to evaluate the biological activity of extracts derived from various morphological components of *Sida rhombifolia*. The aqueous-methanol extract of the entire plant of *Sida rhombifolia* demonstrated significant antibacterial efficacy in an in vitro activity test. The methanolic extract derived from the fruit of this organism exhibited noteworthy antibacterial activity in vitro against various kinds of bacteria. According to reports, the efficacy of leaf extract in mitigating nephrotoxicity and renal dysfunction generated by GM has been proven in an animal model research [7-9].

In a separate investigation, the anti-inflammatory effect of the methanolic extract derived from the aerial section of the subject was examined in an animal model. The utilization of ethanol and aqueous extracts derived from the aerial sections of the plant has been documented as beneficial for the management of arthritis [10]. This finding suggests that *Sida rhombifolia* has the potential to serve as a valuable source of natural antioxidants. Recently, comparable findings were also documented in animal models by the utilization of root and stem extracts. The administration of an aqueous extract derived from leaves was conducted on rats with hyperbilirubinemia, revealing promising potential of this plant as a novel source of medications for those affected by hyperbilirubinemia [11, 12].

The stem of *Sida rhombifolia* was subjected to Soxhlet extraction using methanol as the solvent. The chemical was isolated from *Sida rhombifolia* for the first time, as noted in the study. The chemical exhibited significant antibacterial activity against multiple strains of bacteria in an in vitro test. Nevertheless, there is a lack of documented research about the assessment of the antibacterial properties exhibited by chemicals derived from the roots of this plant. In this investigation, we endeavored to extract and describe compounds derived from the root components of *Sida rhombifolia* Linn [13-15]. Additionally, we aimed to assess the antibacterial properties of these compounds.

MATERIAL AND METHODS

Commonly used solvents in laboratory settings include petroleum ether, chloroform, ethyl acetate, acetone, methanol, and distilled water, which are employed for purposes like as extraction and column elution. Chromatographic studies were conducted using aluminum sheets that were pre-coated with silica gel. The identification of compound spots on thin-layer chromatography (TLC) plates was accomplished through the utilization of ultraviolet (UV) light and iodine vapor. The process of solvent evaporation was conducted utilizing a rotary evaporator, while extraction was facilitated by employing a HY-5A Manoeuvre style vibrator. The antibacterial activity test utilized a conventional antibiotic disc and culture media. The H-NMR, 13C-NMR, and DEPT-135 spectra were acquired using a Bruker Advance 400 MHz spectrometer. The solvent employed for all spectroscopic analyses was CDCl3. In this study, infrared spectra were acquired using a Perkin-Elmer BX infrared spectrometer, with potassium bromide (KBr) as the sample matrix. The melting point determination was conducted using a Griffin melting point instrument.

Collection and Extraction

The plant material was subjected to a drying process at ambient temperature, ensuring it was not exposed to direct sunlight. Subsequently, the desiccated substance was subjected to mechanical grinding using a specialized apparatus. A total of 100 grams of powdered root material was subjected to sequential extraction utilizing the maceration technique. The filtrate samples were concentrated under reduced pressure using a rotary evaporator and afterwards underwent an antibacterial activity test. Upon conducting a comparative analysis of the antibacterial properties exhibited by the crude extracts derived from the aforementioned solvent systems, it was determined that the chloroform extract displayed the most promising results. Consequently, the decision was made to proceed with the chromatographic isolation of the contents present within the chloroform extract. Subsequently, a significant quantity of the powdered substance underwent extraction using identical methodology, utilizing two distinct solvent systems.

Assessment of Antibacterial Property

The antibacterial activity studies utilized the following test organisms: Staphylococcus aureus ATCC25903, Escherichia coli ATCC25722, Pseudomonas aeruginosa DSMZ1117, and Salmonella thyphi. ATCC13311.

Disk Diffusion Antimicrobial Assay and Test Solution Preparation

The test solutions were produced by dissolving 100 mg of each of the crude extracts in 1 ml of dimethyl sulfoxide (DMSO), resulting in a final stock concentration of 100 mg/ml for each test sample. The bacterial suspension was evenly distributed onto the 90 mm Petri dishes containing Mueller Hinton agar by means of a sterilized cotton swab. Subsequently, sterile discs with a diameter of six millimeters were positioned on the surface of the inoculated Agar in Petri dishes. Following this, 50 microliters of each test solution were administered onto the discs. Following the addition of test solutions onto the discs, the extract was subjected to a diffusion process for a duration of 5 minutes. Subsequently, the plates were placed in an incubator set at a temperature of 37°C for a period of 24 hours [16, 17].

Isolation and Characterization

To isolate chemicals from *Sida rhombifolia* root chloroform extract, silica gel column chromatography was used. A glass column contained 100 g petroleum ether-dissolved silica gel slurry. The crude substance was dried on silica gel. The solvent was allowed to evaporate, and the dry sample adsorbed on silica gel was placed in the column. TLC studies of crude material separated pigments well on TLC plate in petroleum ether and ethyl acetate solvent system. A total of 123 40-ml portions were collected. Rotary evaporators extracted fraction solvents with decreasing pressure. Exposure to iodine vapor and UV light at 254 and 365 nm revealed TLC plate spots. The fractions with similar TLC development characteristics were mixed and dried under decreased pressure using rotary evaporators [18, 19].

RESULTS

Scientific investigations have demonstrated that extracts derived from various components of the plant, especially its roots, exhibit diverse biological activities, including antibacterial properties. These findings imply that the plant has promise as a possible source for the development of novel antibacterial medications. This study focuses on the extraction and isolation of compounds derived from the roots of *Sida rhombifolia*, as well as the subsequent evaluation of the antibacterial properties of these isolated compounds.

Evaluation of *Sida rhombifolia* Root Crude Extracts and Isolated Chemicals for Antibacterial Activity

For preliminary antibacterial activity tests, 100 g of plant material was macerated in petroleum ether chloroform, methanol, and gradient extractions. 1.62 g, 2.04 g, and 1.9 g of crude material were obtained from petroleum ether, chloroform, and methanol filtrates after solvent removal. The extracts were tested for antibacterial activity against four microorganisms using the preceding methods. The crude extracts were tested for antibacterial activity against each test bacterium species in duplicates. Results are presented as the average mean ± standard deviation of the observed inhibitory zones [20, 21].

The three crude extracts had considerable antibacterial activity against all bacterial species, however they were lower than the reference substance. The results support previous publications on *Sida rhombifolia* methanolic root extract's antibacterial properties and traditional use of its roots to treat bacterial infections and wounds. The data showed that methanol extracts are more effective than chloroform and petroleum ether extracts against E. coli and S. typhimurium.

Isolated *Sida rhombifolia* Root Compounds Characterization

The three distinct chemical components (referred to as SRL-1, SRL-2, and SRL-3) obtained from the chloroform extract derived from the root of *Sida rhombifolia* were identified as n-hexacos-11-enoic acid, stigmasterol, and β-sitosterol, respectively (as depicted in Figure 1). The compounds were characterized utilizing spectroscopic methodologies, specifically NMR (Nuclear Magnetic Resonance) and IR (Infrared) spectroscopic techniques. The determination of the compound's structure was achieved through a comparative analysis of the observed spectrum and melting point data with the documented data of these compounds in existing literature [22, 23].

Table 1: Bacterial inhibition zones of 50 mg/ml *Sida rhombifolia* root crude extracts

Extracts		Bacterialspecies		
	E.coli	***S.aureus***	***P.aeruginosa***	***S. typhimurium***
PEextract	15± 2	15± 3	11± 1	15.6±0.3
Chl.extract	16± 1	17± 2	12.6± 2	13.6±0.2
Met.extract	21± 2	16± 4	17± 3	16.3±4
Ciprofloxacin	36	33	29	31

Structure Elucidation of SRL-1

The chemical SRL-1 exhibits colorless crystalline properties with a measured retention factor (Rf) value of 0.6. The infrared (IR) spectra of the sample, specifically potassium bromide (KBr), exhibited absorption bands corresponding to the carboxylic acid hydroxyl group at a wavenumber of 3407 cm-1, the carbonyl group at 1705 cm-1, and the long aliphatic chain at 731 cm-1. The substance in question is most likely an aliphatic acid. The supplementary material 2 has the 1 H-NMR spectra of SRL-1, which indicates the presence of olefinic groups at chemical shifts of δ5.38 and 5.34 for H-11 and H-12, respectively. Two doublets seen at chemical shifts of δ2.82 and 2.79 are indicative of the presence of methylene protons adjacent to a carboxylic group on the carbon atom labeled as C-2.

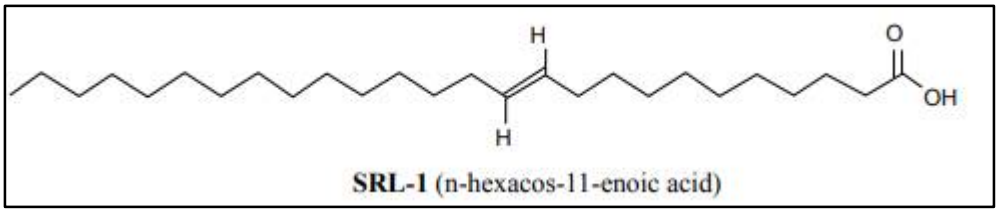

Fig 1: The hypothesized structure of *Sida rhombifolia* (SRL-1)

Structure Elucidation of SRL-2

The chemical was acquired in the form of a white powder, with a retention factor (Rf) value of 0.33. The infrared (IR) spectra of SRL-2, as observed using potassium bromide (KBr), does not exhibit a doublet band in the vicinity of 2850 and 2750 cm-1. This absence of a doublet band suggests that the chemical does not possess an aldehyde functional group. The lack of a prominent peak within the region of around 1700-1800 cm-1 further substantiates the absence of a carbonyl group within the molecule. The lack of discernible bands within the spectral region of 2000 to 1650 cm-1 suggests the absence of any aromatic functional groups within the molecule. Conversely, the detected stretching band with a wavenumber of 3429 cm-1 suggests the existence of a hydroxyl functional group. Based on the analysis of the infrared data, it may be inferred that the chemical under investigation potentially exhibits characteristics of an alcohol compound containing a carbon-carbon double bond within its molecular structure.

Structure Elucidation of SRL-3

The chemical was acquired in the form of a colorless solid with needle-like morphology, with an Rf value of 0.30. The infrared (IR) spectra of SRL-3, specifically using potassium bromide (KBr) as the medium, revealed the absence of a doublet band in the vicinity of 2850 and 2750 cm-1. This absence signifies the absence of an aldehyde functional group within the compound. The lack of a prominent band provides evidence that the molecule does not possess a carbonyl group. The lack of discernible bands within the 2000 to 1650 cm-1 range suggests the absence of any aromatic functional groups within the molecule. Therefore, the detected stretching band at a wavenumber of δ3432 cm-1 provides evidence for the existence of a hydroxyl functional group. The prominent peak observed at a wavenumber of δ3007 cm-1 corresponds to the stretching vibration of carbon-hydrogen (C-H) bonds in alkenes. Conversely, the peaks observed at wavenumbers δ2930 and δ2855 cm-1 are indicative of the stretching vibrations of C-H bonds in methylene and methyl groups, respectively.

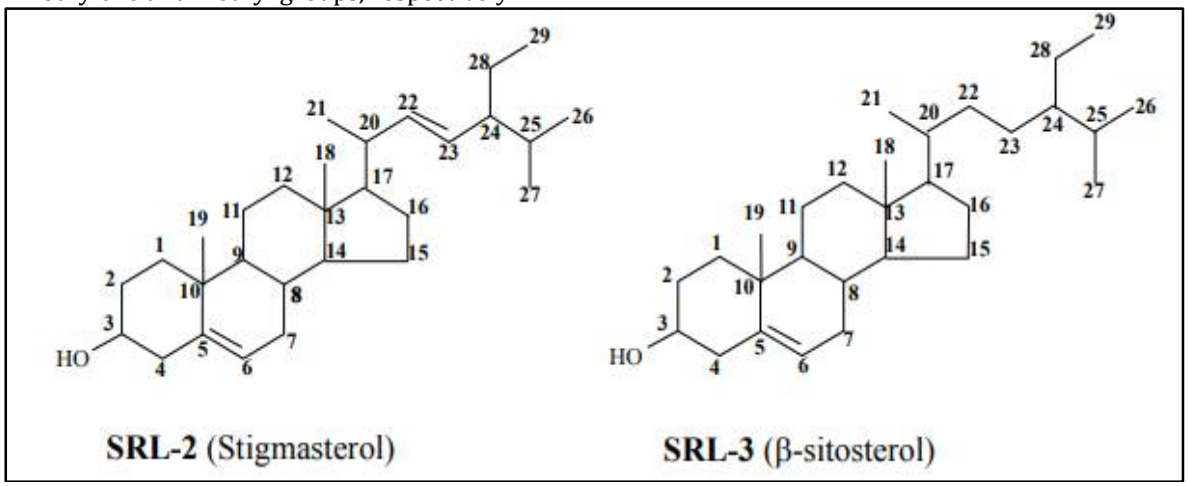

Fig 2: The hypothesized structure of *Sida rhombifolia* root compounds SR-2 and SR-3

Antibacterial Test of the Isolated Compounds

The antibacterial properties of the isolated compounds (SRL-1, SRL-2, and SRL-3) were assessed through in vitro experiments utilizing the Agar diffusion method. Four bacterial species, namely E. coli, S. aureus, P. aeruginosa, and S. typhimurium, were utilized for this evaluation. The chemicals' actions were quantified by measuring the zones of growth inhibition. The inhibitory actions of the substances in terms of growth are provided. The antibacterial activity of the separated compounds exhibited reduced efficacy compared to the reference medication, similar to the crude extracts [24-26].

Table 2: ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of SRL-3

Sr. No	Compounds	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhimurium*
1	SRL-1(*n*-hexacos-11-enoicacid)	12	13	10	12
2	SRL-2 (Stigmasterol)	13	13.0	10.0	12
3	SRL-3(β-sitosterol)	14.0	12	9.6	13
	Ciprofloxacin	36	33	29	31

Furthermore, the compounds exhibited minimal antibacterial efficacy against Pseudomonas aeruginosa. As demonstrated previously, SRL-1 exhibited antimicrobial efficacy that was similar to that of SRL-2 and SRL-3. Nevertheless, a thorough examination of existing literature reveals a dearth of studies that may be utilized for the purpose of comparing and contrasting our findings. The antimicrobial properties of SRL-2 exhibited a moderate level of effectiveness when compared to the reference medication. This observation is consistent with earlier studies that have demonstrated the limited antibacterial efficacy of stigmasterol against Acetobacter sp., E. coli, S. aureus, Streptococcus sp., and P. aeruginosa. In contrast, the observed antibacterial activity aligns with existing literature findings regarding the relatively low to moderate bactericidal effects of β-sitosterol against several bacterial strains, such as S. aureus, E. coli, and P. aeruginosa [27-31].

CONCLUSION

In summary, the crude acetone extract yielded three chemicals, namely SRL-1, SRL-2, and SRL-3. The substances were identified as n-hexacos-11-enoic acid, stigmasterol, and β-sitosterol, respectively, through the analysis of their physical properties, spectroscopic data (IR and NMR), and relevant literature sources. This study presents the initial report on the isolation of SRL-1 from *Sida rhombifolia*. The in vitro test findings demonstrated that the separated compounds exhibited antibacterial activity that were comparatively lower than those of the reference compound. When comparing the compounds, their antibacterial properties were found to be similar. However, the efficacy of the compounds exhibited substantially lower activity against Pseudomonas aeruginosa. The findings were also in line with those obtained from the crude extracts. The antibacterial activity reported in both the crude extract and isolated components provide support for the traditional medicinal usage of the plant in treating various bacterial illnesses. Therefore, it is advisable to conduct additional testing on a substantial number of bacterial strains in order to determine their potential as candidates for the creation of antibacterial medications.

REFERENCES

1. Rohman, A., Ikhtiarini, A. N., Setyaningsih, W., Rafi, M., Aminah, N. S., Insanu, M., & Windarsih, A. (2020). *Sida rhombifolia* Linn: Phytochemicals Composition and Biological Activities. *International Journal of Pharmaceutical Research (09752366)*.
2. Kavya, J. B., Murali, M., Manjula, S., Basavaraj, G. L., Prathibha, M., Jayaramu, S. C., & Amruthesh, K. N. (2020). Genotoxic and antibacterial nature of biofabricated zinc oxide nanoparticles from *Sida rhombifolia* Linn. *Journal of Drug Delivery Science and Technology*, *60*, 101982.
3. Kavya, J. B., Murali, M., Manjula, S., Basavaraj, G. L., Prathibha, M., Jayaramu, S. C., & Amruthesh, K. N. (2020). Genotoxic and antibacterial nature of biofabricated zinc oxide nanoparticles from *Sida rhombifolia* Linn. *Journal of Drug Delivery Science and Technology*, *60*, 101982.
4. Debalke, D., Birhan, M., Kinubeh, A., & Yayeh, M. (2018). Assessments of antibacterial effects of aqueous-ethanolic extracts of *Sida rhombifolia*'s aerial part. *The Scientific World Journal*, *2018*.
5. Membe Femoe, U., Kadji Fassi, J. B., Boukeng Jatsa, H., Tchoffo, Y. L., Amvame Nna, D. C., Kamdoum, B. C., ... & Dimo, T. (2022). In vitro assessment of the cercaricidal activity of Sida acuta Burm. F. and *Sida rhombifolia* Linn.(Malvaceae) hydroethanolic extracts, cytotoxicity, and phytochemical studies. *Evidence-Based Complementary and Alternative Medicine*, *2022*.
6. Thounaojam MC, Jadeja RN, Ramani UV, Devkar RV, Ramachandran AV (2011) SiSida rhomboidea. Roxb Leaf Extract Down-Regulates Expression of PPARγ2 and Leptin Genes in High Fat Diet Fed C57BL/6J Mice and

Retards in vitro 3T3L1 Pre-Adipocyte Differentiation. Int J Molc Sci 12: 4661-4677.
7. Cunha AH, Merétika, Peroni N, Hanazaki N (2010) Local knowledge of medicinal plants in three artisanal fishing communities (Itapoá, Southern Brazil), according to gender, age, and urbanization. Acta Bot Bras 24: 386-394.
8. Singh N, Dubey K (2012) An ethnobotanical study of medicinal plants in Sonebhadra District of Uttar, Pradesh, India with reference to their infection by foliar fungi. J Med Plant Res 6: 2727-2746.
9. Assam AJ, Dzoyem JP, Pieme CA, Penlap VB (2010) In vitro antibacterial activity and acute toxicity studies of aqueous-methanol extract of *Sida rhombifolia* Linn. (Malvaceae). BMC Complement Altern Med 10: 40.
10. Megersa M (2011) Ethnobotanical study of medicinal plants in Wayu Tuka Wereda, East Wollega zone of Oromia region, Ethiopia. Addis Ababa Univeristy, Addis Ababa, Ethiopia.
11. Seshathri K, Thiyagarajan T (2011) Antimicrobial activity of chewing sticks of Jimma-Ethiopia against Streptococcus pyogens. J Phytolog 3: 34-37.
12. Rashmi RS, Uma SM, Prasanta KC (2010) Comparative In vitro Antimicrobial Activity Studies of *Sida rhombifolia* Linn fruit extracts. Inter J Pharm Tech Res 2: 1241-1245.
13. Thounaojam MC, Jadeja RN, Patel DK, Devkar RV, Ramachandran AV (2010) Acute and sub chronic oral toxicity of Sida rhomboidea.Roxb leaf extract. J Complementary and Integrative Medicine 7:1.
14. Singh A, Malhotra S, Subban R (2008) Anti-inflammatory and analgesic agents from Indian medicinal plants. Intl J of Integrative Biol 3: 57-72.
15. Gupta SR, Nirmal SR, Patil RY, Asane GS (2009) Anti-arthritic activity of various extracts of *Sida rhombifolia* aerial parts. Nat Prod Res 23: 689-695.
16. Ranjan SR, Shankar MU, Kumar PS, Saiprasanna B (2011) Evaluation of antidiarrhoeal activity of *Sida rhombifolia* Linn. root. Intl Res J Pharm 2:157- 160.
17. Bumrela SB, Naik SR (2011) Identification of β-carotene and β-sitosterol in methanolic extract of Dipteracanthus patulus (Jacq) nees and their role in antimicrobial and antioxidant activity. Intl J Phytomedicine 3: 204-215.
18. Narendhirakannan RT, Limmy TP (2010) In vitro antioxidant studies on ethanolic extracts of leaf, stem and root of *Sida rhombifolia* L. Intl J Pharma and Bio Sci 2: 1-10.
19. Mohd FF, Kadir HA, Tayyab S (2012) Bilirubin clearance in temporarily hyperbilirubinemic rats treated with aqueous extract of *Sida rhombifolia*. Life Sci J 9: 2254-2256.
20. Dhalwal K, Vaibhav MS, Singh B, Kakasaheb RM (2010) Hypoglycemic and hypolipidemic effect of *Sida rhombifolia* ssp. retusa in diabetic induced animals. Intl J Phytomed 2: 160-165.
21. Poojari R, Gupta S, Maru G, Khade B, Bhagwat S (2009) *Sida rhombifolia* ssp. retusa seed extract inhibits den induced murine hepatic preneoplasia and carbon tetrachloride hepatotoxicity. Asian Pac J Cancer Prev 10: 1107-1112.
22. Yan-Hong W, Bharathi A, Jadhav AN, Smillie TJ, Khan IA (2008) Structural characterization and identification of ecdysteroids from *Sida rhombifolia* L. in positive electrospray ionization by tandem mass spectrometry. Rapid Commun Mass Spectrom 22: 2413-2422.
23. Jadhav AN, Swamy RC, Bharathi A, Khan IA (2007) HPTLC method for determination of 20-Hydroxyecdysone in *Sida rhombifolia* L and dietary supplements. Chromatographia 66: 797-800.
24. Chowdhury A, Ashraful Alam M, Rahman MS, Rashid MA (2009) Phytochemical and biological investigations of Sida Rhomboidea Linn. Internet J Alternative Med 7
25. Menaka TC, Jadeja RN, Devkar RV, Ramachandran AV (2010*) Sida rhomboidea*.Roxb leaf extract ameliorates gentamicin induced nephrotoxicity and renal dysfunction in rats. J Ethnopharmacol 132: 365-367.
26. Muthuswamy R, Solomon MA (2009) Ethinomedical survey of folk drugs used in Bahirdar Zuria district, Northwestern Ethiopia. Ind J Trad Knowl 8: 281-284.
27. Wayne PA (2009) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 9th edition, Clinical and Laboratory Standards Institute.
28. Sharma SK, Vasudev N, Ali M (2009) A new aliphatic acid from Achyranthes aspera Linn Roots. Ind J Chem 48: 1164-1169.
29. Pateh UU, Garba HM, Iliya I, Sule IM, Abubakar MS, et al. (2009) Isolation of stigmasterol, β-sitosterol and 2-hydroxyhexadecanoic acid methyl ester from the rhizomes of *Stylochiton lancifolius* pyer and kotchy (araceae). Nig J Phar Sci 7: 19-25.
30. Sahidin A, Muhammad T, Marianti M (2011) Terpenoids from the stem bark of jatropha plants and their biological activities. MAKARA SAINS 15: 106-110.
31. Kamboj A, Kumar AS (2011) Isolation of stigmasterol and β-stosterol from petroleum ether extract of aerial part of Ageratum conyzoides (Asteraceae). Intl J Pharm Pharmaceut Sci 3: 94-96.

CITATION OF THIS ARTICLE

Ankit Kumar Saini, Arun Nivas Velumani, Hari V., Suhas Narayan Sakarkar, Brijesh Kumar Singh, Mohammad Rizwan, Abhishek Amod Gupta. Antibacterial Activity of Compounds Extracted from *Sida rhombifolia* Linn. . Bull. Env. Pharmacol. Life Sci., Spl Issue [2]: 2023: 495-500.