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"Free radical scavenging by DPPH method of Annona muricata ethanolic leave extract with metallic conjugates and its anti- bacterial activity"

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ABSTRACT

Background: The aim of this study is to analyze the antioxidant properties and antibacterial efficacy of 'Soursop' A. muricata L. ethanolic leave extract with its Iron and Copper nanoparticles against gram negative and gram positive bacteria. A. muricata L. extract-mediated green synthesis proposed in this work is a fast, economical and effective technique to synthesize NPs with promising antibacterial activity a well published work done by (**Vidhya. S, et al., 2022**)

Methods: In this study, we used known gram positive (Staphylococcus aureus and Streptococcus mitis) and gram negative bacteria (Escherichia coli and Klebsiella pneumoniae) to analyse the Antibiotic susceptibility testing by using 'Disc diffusion method' and 'Agar well diffusion' method.

Experiments performed: Known bacterial culture was used on different agar medium like NAM, MHA, McC, for anti-microbial susceptibility testing of 'Soursop' leaves extract and its conjugates (FeNP & CuNP) compared with known antibiotics.

Result: Ethanol extract of Annona muricata L. showed good antibacterial activity. Among the selected solvents and prepared metallic conjugates with $FeCl_3$ and $CuSO_4$ all of these exhibited significant (mild to moderate) antibacterial effect on selected known bacterial strains by using Nutrient Agar and similar zone were measured on Muller-Hinton agar. Charcoal filter (CH) extract shows moderate effect against selected Gram positive & negative bacteria.

Conclusion: In our study we used ethanolic extraction and aqueous ethanolic 50% EtOH in another group as per modified method adapted from (**A. Hussain, et al., 2019**) the aqueous ethanolic extraction shows maximum yield as it extracted water as well as alcohol soluble phyto-compounds of A. muricata leaves. The disk diffusion method & Agar well diffusion method was appropriate only as a preliminary screening

test prior to MIC determination with the dilution method. S. aureus and E. coli were the most tested microorganisms belong to Grams positive & negative category.

Key words: Annona muricata L, Gram positive & negative bacteria, nanoparticles, antioxidant.

1. INTRODUCTION:

Plants have provided man all his basic needs and also important medicines. Many traditional medicine systems formed by the plants among which are Ayurvedic, Unani, Chinese and others. Antioxidant activity of plants used to limit the oxidation of various molecules by blocking oxidative chain reactions. Primary antioxidants scavenge free radicals directly. Antimicrobial Susceptibility Testing (AST) is done to find out the efficacy of pla nt products in antimicrobial treatment.

Annona muricata:

A. muricata, also called guanabana, soursop, graviola, or Brazilian paw paw, is a plant native to Central America. It is widely found in Southeast Asia, South America, and African rainforests. A. muricata belongs to the family Annonaceae. (**N. Bhattacharjee, et al., 2022**)

Ethanolic leaf extract contains phosphorous and iron. The root extract has the highest calcium content .The leaf extract also exhibits strong antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus. (**C. Vijayameena et al., 2013**)

In the current study the solvents used for plant extraction were aqua- ethanolic. The antibacterial activity was tested using the Kirby-Bauer disc diffusion method. Both extracts showed antibacterial properties, with the ethanolic extract being more effective against various organisms. Escherichia coli were the most susceptible Gram-negative bacteria. The study suggests that Annona muricata can help treat diseases caused by these organisms and may work well with antibiotics for better results. (**N. Kamath et al., 2017**)

Antioxidants help reduce free radicals in the body and may prevent diseases. Soursop leaves are traditionally used for cancer prevention and as a natural antioxidant, often consumed as a boiled tea. Many studies conducted on the phytochemical content of fresh boiled water, fresh juice, and fresh extract of Soursop leaves, and antioxidant activity using DPPH with the help of UV-Vis spectrophotometer. The ethanol extract had the good antioxidant activity compared to the fresh decoction and juice study conducted by. (**Eva Fransiska et al., 2023**)

2. MATERIALS AND METHODS:

Collection of Plant sample: The Soursop leaf (Annona muricata) was obtained from the trees planted in Madan Mohan Herbal Garden of JNCH & RC, Bhopal in the month of February'2025. After collection and washing of the leaf, they were separated from the twigs and dried in shade for further preparation.

Source of identified bacterial species: Both gram positive & negative bacterial strains were collected from Department of Microbiology, JNCH & RC, Bhopal.

Preparation of extracts: Ethanolic extracts of the Soursop leaf were prepared in the Pharmacology lab, Department of Research & Clinical Genetics, JNCH & RC, Bhopal, India. Maceration method is used for

extraction as it is a convenient, simple, inexpensive, and favourable technique, especially in the case of small-scale extraction, such as that at laboratory scale. 45 gram of leaves powder were used in extraction process and their percent yield was calculated and solubility test were performed.

Standard Antibiotics used: Ampicillin 10 mcg, CO- trimoxazole 25 mcg, Piperacillin / Tazobactam 100/10 mcg, Cefuroxime CXM 30 mcg, Miconazole MIC 50, Amoxyclav AMC 30, Ceftazidine CAZ 30 mcg, Gentamycin 10 mg/ml, Penicillin / Streptomycin 10 mg/ml, Penicillin / Streptomycin / Neomycin 10 mg/ml, Colistin CL10 mcg.

Free Radical Scavenging Activity by DPPH Method: Potential of I. pes-caprae extracts to scavenge DPPH radical was determined as per (**Mensor et al., 2001)** and **(Manigauha et al., 2009)**. Stock solution of all the extracts and swaras (1.0 mg/ml) was diluted to 25, 50, 75, 100 and 125 µg/ml. Two ml samples solution of different concentrations was added to 1.0 ml of 0.3 mM DPPH in ethanol and 2.0 ml of Phosphate buffer (0.2 M, pH 7.4). The reaction mixture was incubated for 30 min in dark at room temperature. After 30 minutes the absorbance was measured by spectrophotometer (UV-Vis- 2202TS spectrophotometer- Systronics) at **517 nm.** The control solution was prepared containing water instead of extract and blank was prepared without the addition of DPPH. Ascorbic acid was used as a standard. The scavenging activity (%) was calculated using the following formula:

Inhibition Percentage (I %) = (Ac – At/Ac) X 100

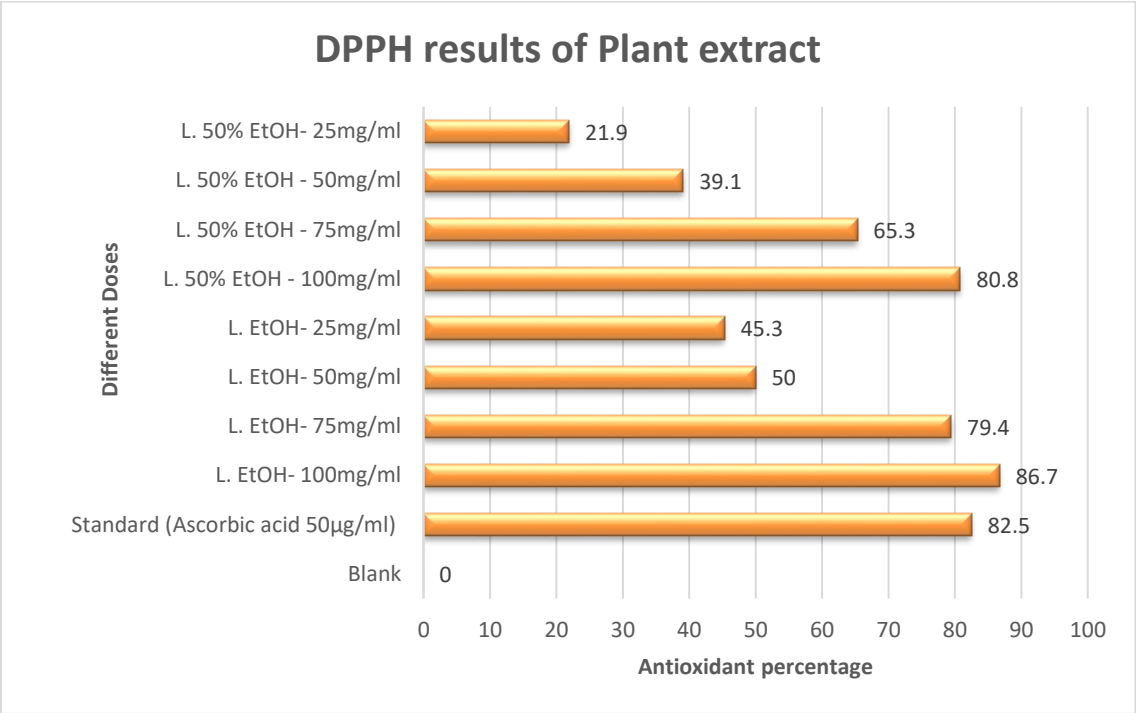
Where **Ac** is the absorbance of the control and **At** is the absorbance of the test compound and observation were recorded

Methods for Antimicrobial susceptibility testing: (Mounyr. B et al., 2016)

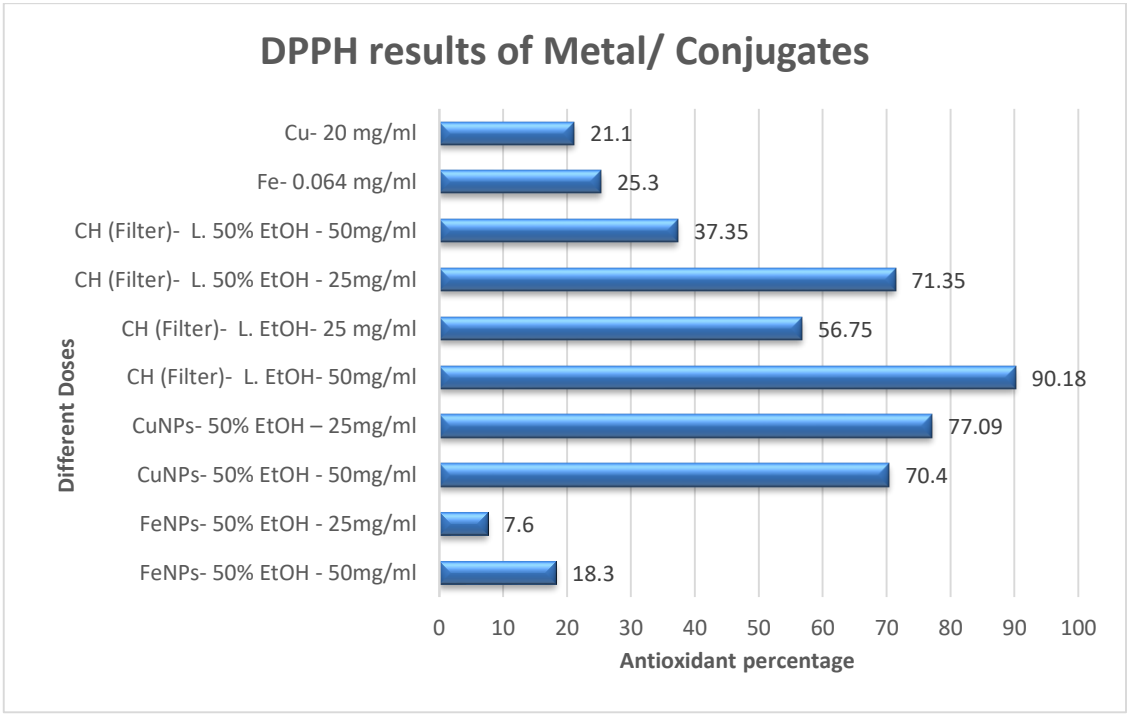
- Disc diffusion or ‘Kirby-Bauer’ method.
- Agar well diffusion method.

Microscopic analysis: Motic BA-210 microscope with imagining system with 40 to 100 X magnification was used.

3. OBSERVATION:

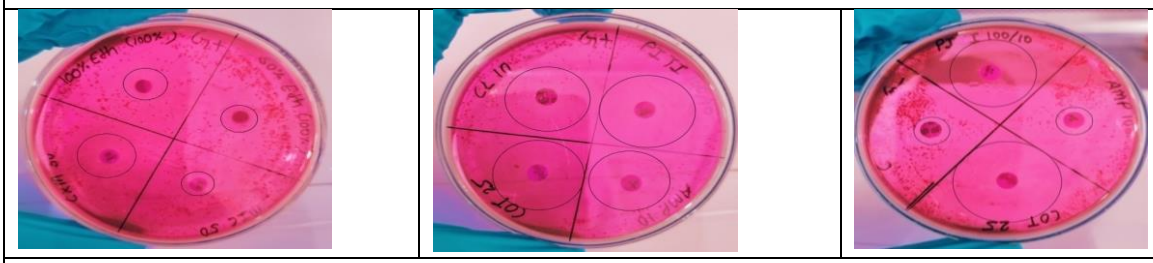


Graph: 01 Showing antioxidant percentage of ethanolic leaves extract of Annona muricata by DPPH method with Ascorbic acid as standard.

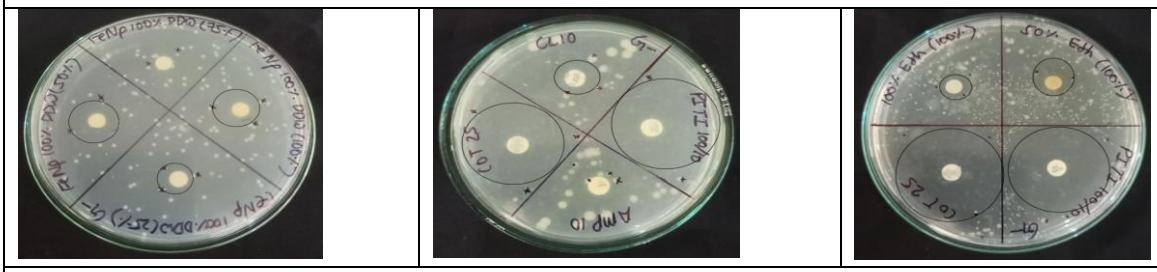


Graph: 02 Showing antioxidant percentage of metallic conjugates of ethanolic leaves extract of Annona muricata by DPPH method.

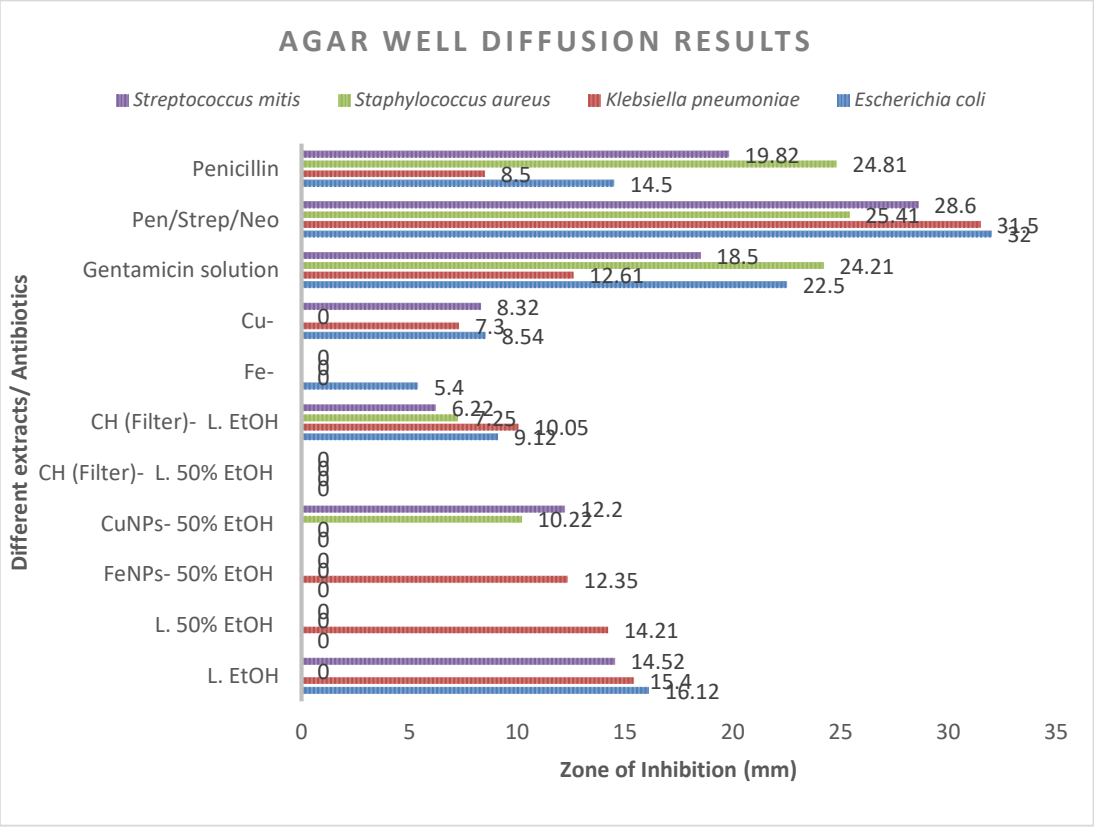
Photograph: 01
Showing 'Zone of inhibition' (mm) on **Nutrient Agar** by using different extracts of **Annona. M** against different Gram Positive (**+ive**) and Gram Negative bacteria by Disc diffusion method.



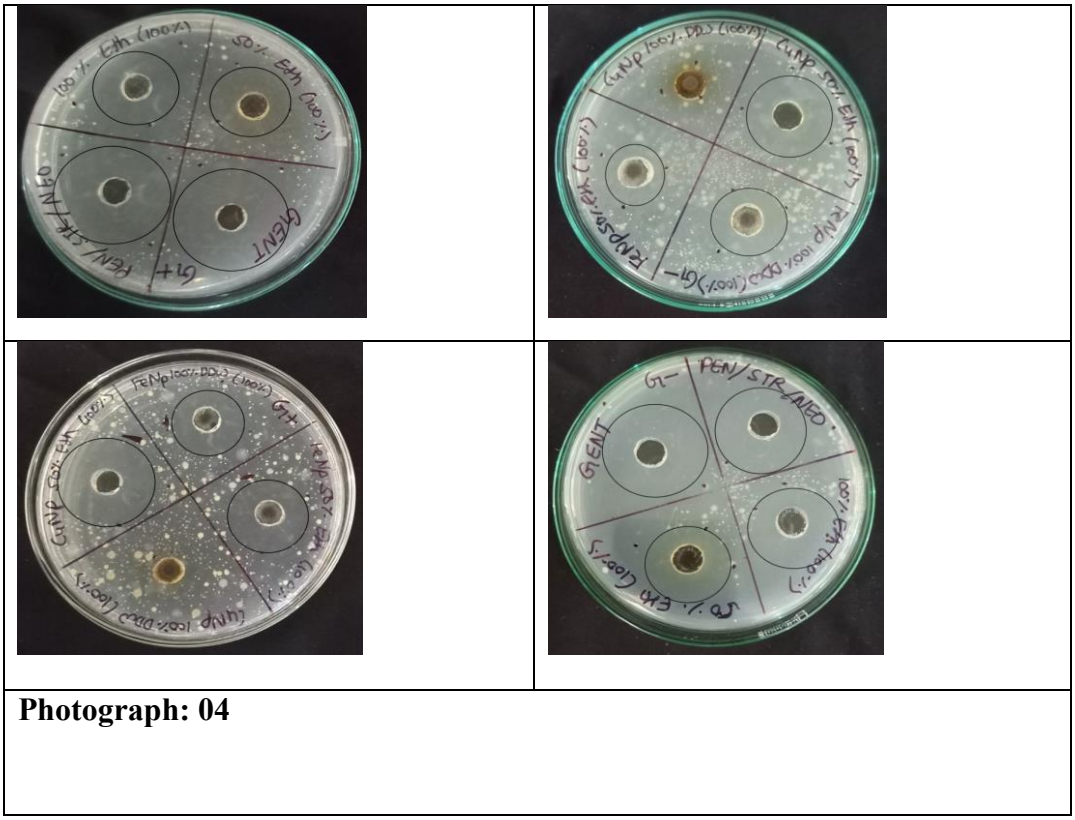
Photograph: 02
Showing 'Zone of inhibition' (mm) on **MacConkey agar** (MAC) by using different extracts of **Annona. M** against different Gram Negative (**-ive**) bacteria by **Disc diffusion** method.



Photograph: 03
Showing 'Zone of inhibition' (mm) on **Mueller-Hinton Agar** (MHA) by using different extracts of **Annona. M** against different Gram Positive (**+ive**) and Gram Negative bacteria by **Disc diffusion method**.



Graph: 03 Showing 'Zone of inhibition' (mm) on **Mueller-Hinton Agar** (MHA) against different Gram Positive (**+ive**) and Gram Positive **(-ive**) bacteria by **Agar well diffusion** method.



Photograph: 04

Showing MHA plates loaded with different Antibiotics, leave extract and Metallic conjugates (NPs) Zone of inhibition by **Agar well diffusion method**

4. RESULT:

In present study, we observed that ethanolic leave extract of A. muricata have good susceptibility against Staphylococcus aureus and Streptococcus mitis which is higher than the inhibitory activity of standard control antibiotic like AMP 10 and PIT 100/10 on Nutrient Agar medium by (Disc diffusion method) similar zone were measured on Mueller Hinton agar, and the prepared conjugates FeNPs and CuNPs shows moderate inhibitory effect on Staphylococcus aureus and Streptococcus mitis, The activated charcoal filtered extract shows mild effect these against Gram positive bacteria, Best captured zone of inhibition clearly shown in (Photograph: 1,2 &3).

The prepared nanoparticles or metallic conjugates shows good inhibitory effect on bacterial growth as compare to pure ethanolic extract of Annona muricata L, as these extract contains a wide spectrum of activity against common bacteria that are responsible for the most of the bacterial diseases concluded by (**A. Hussain, et al., 2019**). Thus, the plant leaves has an abundant of the antibacterial compounds as elevated results are observed with increased concentration and its ethanolic extracts exhibited significant antibacterial activities on the tested bacteria Mean ± SD values of zone of inhibition (mm) shown in (Photograph: 04). Our study result was relevant as compare to the result of (**Uchegbhy et al., 2017**), (**Gajalakshmi et al., 2012**) and (**Pathak et al., 2010**). The antibacterial activities may due to strong occurrence of different chemical compounds such as alkaloids, flavonoids, tannins, phenols, steroids and Saponins reported by (**Vidhya. S, et al., 2022**).

DPPH method was performed with some modification outlined by (**Celep et al., 2015**), Ascorbic Acid (50µg/ml) was used as a standard control. By using a UV-Vis spectrophotometer (Systronics) absorbance of the 50 µg/ml DPPH was determined at a wavelength of 517 nm. The DPPH radical scavenging activity was recorded in terms of % Inhibition as shown in (Table: 01) with P value: 0.0051 on statistical significance level (<0.05).

It was observed that 'L. EtOH- 100mg/ml' concentration shows (Mean ± SD; 0.047 ±0.0246) with higher 86.7% antioxidant activity, another test group 'L. 50% EtOH - 100mg/ml' (Mean ± SD; 0.642 ±0.108) with good 80.8% antioxidant activity. Alone metals have minimum DPPH scavenging activity (lesser than 26%) and its conjugate with extract 'CuNPs- 50% EtOH - 0.03mg/ml' (0.105 ±0.0327) shows 70.4% activity all the results obtained in triplicates and statistically significant with $p<0.05$.

5. CONCLUSION AND DISSCUSSION:

In our study we used ethanolic extraction and in another group as reviews about aqua-ethanolic extraction (50% EtOH) shows it's capabilities of extracting many phytochemicals which are water as well as alcohol soluble. Several different classes of metabolites were reported to exist in the extract of A. muricata, such as tannins, alkaloids, flavonoids, polyphenols, saponins, acetogenin and also contain triglycosides, megastigmans (**Moghadamtousi et al., 2015**). According to (**Klancnik, et al., 2010**), the disk diffusion method & Agar well diffusion method was appropriate only as a preliminary screening test prior to MIC

determination with the dilution method. S. aureus and E. coli were the most tested microorganisms belong to Grams positive & negative category.

Clinical trials investigating the vast pharmacological potential and antibacterial properties of A. muricata are another area that has been disregarded but now requires close attention. The overall activity of this plant occurs due to the presence of acetogenin, which is the most abundant chemical family in various parts of A. muricata. In the present study, the zone of inhibition by disc diffusion & Agar well diffusion method was carried out to measure the anti bacterial effect against selected gram positive & gram negative bacteria (**Silva, D, et al.,2019**).

6. FUTURE PROSPECTIVE:

A. muricata is extensively used in traditional medicine to treat a myriad of conditions such as hypertension, diabetes and cancer. Decoctions of all parts are widely used in preparations. In vitro and in vivo studies support traditional uses of A. muricata but lack of clinical validation. More than 200 phytochemicals have been identified in this plant, mainly acetogenins, alkaloids and phenols. These phytochemicals have shown pharmacological activities such as antimicrobial, antioxidant, insecticide, larvicidal, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti ulceric, wound healing, anti-jaundice, hepato protective, hypoglycemic, immunomodulatory, and antimalarial among others.

The extracts of A. muricata have shown better and promising activity like in non-communicable diseases, be used to help save the human race with very low cost of treatment. Using the natural compounds in their proved form, rather than taking years trying to develop a patentable synthetic analogue as the human race suffers, may be the best service we might ever give to this world by developing cheapest remedies with effective results.

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Author Contributions:

“First authors [Manshi Anand] contributed to the study experimental part. Material preparation, data collection and analysis were performed by [Sarfaraz Hanfi & Alibha Guru Rawat]. The first draft of the

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Not required

Consent to publish:

No photographs and images of any individual used in the manuscript.

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