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CHARACTERIZATION OF BIOACTIVE COMPOUIND ISOLATED FROM STRYCHONUS POTATORIUM USING BACTERIAL PATHOGENS

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ABSTRACT

In this study, phytochemical constituents are extracted from seeds of Strychonus potatorium using low polar to high polar solvents (Hexane, butanol, ethanol, choloroform and aqueous). Active compounds obtained in Strychonus potatorium was analyzed and identified using GC-MS method, it finds out 16-Octadeceonic acid compounds obtained in Strychonus potatorium. The isolated active phyto-constituents could be an alternate way to combat against bacterial diseases and their influence in control of pathogen multiplication in the aspect of microbiome. The higher concentration of extract (100µg/ml) extracts exhibited a pronounced activity against Pseudomonas aeruginosa (21 mm), Proteus vulgaris (19 mm), Citrobacter sp (19 mm), Klebsiella pneumoniae (18 mm), Micrococcus sp (17 mm), Bacillus subtilis (16 mm), Staphylococcus aureus (15 mm), E. coli (14 mm) and Serratia marcescens (5 mm). The minimum inhibitory concentration and minimum bactericidal concentration were found to be 20-40 µg/ml and 80-100 µg/ml respectively for the extracts of S. potatorium against test organisms. In this study confirms S. potatorium extract possess antibacterial activity against a wide range of microbes justify which could its use in traditional medicine as a remedy for the treatment of bacterial diseases.

***Key words*: *S. potatorium*, antibacterial activity, minimum inhibitory concentration and minimum bactericidal concentration.**

I.INTRODUCTION

Strychnos potatorum Linn.(family : Loganiaceae) commonly known as Katakam in Ayurveda, Tettankottai in tamil and Tettamparal in malayalam is a moderate sized tree found in southern and central parts of India, Srilanka and Burma [1]. The ripe fruit is emetic, diaphoretic and alexiteric; it cures inflammation, anemia, jaundice and causes biliousness [2]. In ayurvedic system of medicine, the seeds are used invitiated conditions of kapha and vata, hepatopathy, nephropathy, gonorrhea, gastropathy, bronchitis, chronic diarrhoea, dysentery, renal and vesicle calculi, diabetes, burning sensation, dipsia, conjunctivitis, scleritis, ulcers, some eye diseases etc [3]. Numerous plants used in folk-lore and tribal medical practices for diabetes mellitus in remote villages of India and tribal pockets, are not known to the mainstream medical practitioners. Two such plants *Artemisia*

pallens and *Cassis kleinii* are recently identified and scientifically validated at TBGRI, Palode, Trivandrum [4]. A search for traditional drugs used for diabetes in remote villages and tribal pockets of India could reveal many useful plants. Worldwide, over 1200 species of plants have been recorded as traditional medicine for diabetes. Some of these plants have been evaluated in laboratories and in a number of cases their efficacy has been confirmed, for instance, *Panax ginseng, Opuntia cactus, Tecoma stans, Syzygium cumini etc.,* [5]. Specific chemical constituents of these plants, such as polysaccharides, alkaloids, triterpenoids and xanthones are believed to be responsible for the hypoglycemic effects and they can be related to actions including increased insulin release and increased glucose metabolism in the body periphery, among others [6]. *Strychnos potatorum* seeds have been reported to possess diuretic [7] and antidiarrhoeal [8] activities. Hepatoprotective and antioxidant activities of the seed powder and aqueous extract of *Strychnos potatorum* seeds against CCl_4-induced acute hepatic injury has been reported [9]. Hence in the present investigation planned to findout effect of active compounds isolated from *Strychnos potatorum* seeds by GC-MS methods against bacterial pathogens.

II.MATERIALS AND METHODS

In the present study *Strychnos potatorum* seeds were selected as a plant sample. The plant sample *Strychnos potatorum* seeds were collected from crude drug market, Tiruvallur, Tamilnadu, India and was identified by taxonomist. The collected plant seeds (Photo.1) were immediately transported to the laboratory and the voucher specimen was deposited in Biotechnology laboratory, Department of Biotechnology, St Peter's University, Avadi.

Photo 1. Seeds of*Strychnos potatorum* Linn.

2.1. Preparation of plant sample and experimental design: The plant sample *Strychnos potatorum* seeds were dried in shadow place at room temperature. The samples are extracted with different organic solvents like hexane, butanol, chloroform, ethanol and water. The above extraction made with same solvent 1g / 100ml (W/V) concentration and screened their antimicrobial activity against test bacterial pathogens.

2.2. Compound isolation and characterization: The crude extract was subjected to GC-MS to identify the bio active constituents present in it. The GC-MS analysis is done in the SITRA (South Indian Textile Research and association), Coimbatore, Tamilnadu, India. The analysis was performed using the equipment Thermo GC -

Trace Ultra Ver: 5.0,Thermo MS DSQ II with the capillary column of , DB 5 - MS capillary standard non - polar column which is 30m long, 0.25mm internal diameter with film thickness of 0.25µm. 1µL of sample was taken for analysis. Helium was employed as carrier gas with flow of 1ml per minute. The oven temperature program was initially set as 70ºC and raised up to 260ºC at 6ºC per minute. The run time of the sample is 37 minutes. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operated in scan mode from 50 to 650 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST.

2.3. Anti bacterial testing: Selected microorganisms include bacteria such as *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Citrobacter* sp, *Klebsiella pneumoniae*, *Micrococcus* sp, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* and *Serratia marcescens* isolated from different pathologic medium from patients diagnosed to have various wound infection at the laboratory Joys Clinical Lab, Manvalanagar, Thiruvallur (district) Tamilnadu. The antibacterial activities of *S.potatorium* extracts were evaluated *in vitro* by a disc diffusion method using Muller Hinton medium.

2.4. Disc diffusion method: Filter paper disc diffusion technique in agar [10] was employed for determining antimicrobial activity. Whatman No.1 filter paper discs of 6mm diameter, placed in dry Petri plates, were autoclaved. Sterile filter paper No.1 discs were loaded with the test extracts by Irobi *et al.*, method [11]. The amount of extracts loaded in each disc, similarly discs were prepared with standard antibiotic streptomycin (w/v) in two different concentrations (20µg/ml and 40µg/ml). The pathogenic strains were suspended in Muller Hinton broth (Hi Media) by transferring a loop full of grown 24h on agar slopes. The suspensions were vortexes and 0.1ml aliquots were spread over respective agar medium plates. The extract extracts and streptomycin loaded discs were then placed over the plates seeded with respective microorganisms. The plates were incubated at 37ºC for 12-24h. The antibacterial activity was determined by measuring the inhibition zone around the discs.

2.5. MIC and MBC test: Minimum inhibition concentration (MIC) of the extracts was determined from the culture plate that had the lowest concentration and prevented and growth of bacterial strains. Minimum bacterial concentration (MBC) was determined by using the method of Samy and Ignacimuthu [12]. The test containing 3ml of Muller Hinton broth and 0.1ml of bacterial suspension and 0.1ml extract extract were incubated at 37ºC for 24hrs. Bacterial turbidity was measured at 650nm to determine the rate of inhibition of bacterial growth. Streptomycin at 20 and 40µg/ml was used as a reference for determination of minimum bactericidal concentrations. The tubes containing only the growth medium and each of the organisms were used as control. The minimum bactericidal concentration that showed reduction of the bacterial growth as measured from the turbidity of the culture assay optical density value.

III.RESULT AND DISCUSSION

The extract sample was isolated and characterized by GCMS method. GC-MS analysis was performed to identify the different compounds present in the crude extract of *A.lanata* leaves. Thirty compounds were identified in GC-MS by library search tools. Thirty compounds were screened for bioactive compounds. One compound 16- Octadecenoic acid is effective compared to other compounds (Fig 1). In this study the

hydrocarbon chain is lengthy, it shows hydrophilicity is reduced and the lipophilicity is increased. Increased lipophilicity is an evidence for transportation of phytomolecules across the epithelial cells. It seems aromatic compounds comprising of aromatic carboxylic acids and esters are also responsible for inhibition of pathogen. Similar kind of finding observed in the antimicrobial activity of *Sansevieria roxburghiana* by Deepa *et al.,* [13].

The isolated compound was found to be effective against all tested organisms with inhibition zone ranging from 5 to 21 mm in the concentration of 100µg/ml. When the result was compared with standard antibiotic Streptomycin a moderate efficiency was observed (Table-1). The extract *S.potatorium* showed highest inhibition activity against *Pseudomonas aeruginosa* (21 mm) followed by *Proteus vulgaris* (19 mm), *Citrobacter* Sp (19 mm), *Klebsiella pneumoniae* (18 mm), *Micrococcus* sp (17 mm), *Bacillus subtilis* (16 mm), *Staphylococcus aureus* (15 mm), *E. coli* (14 mm) and *Serratia marcescens* (5 mm).

Chemical studies performed in different compounds such as amino acids with antibacterial activity are *Lissoclinum cf. badium* [14], *Halocynthia aurantium* [15] and *Styela clava* [16]. Similarly in this study the active compound 16-Otadecenoic acid inhibit remarkable amount in pathogenic organisms. Crude extracts of plants, *A. lanata* has flavonoids, carbohydrates and proteins but lacks tannins, alkaloids, glycosides, terpenoids, saponins and resins [17]. Koperuncholan *et al.,* [18] reported that the ethyl acetate extract of the whole plant extract had tannins, flavonoids, saponins, terpenoid, carbohydrates and alkaloids but resins, proteins and glycosides were absent. In preliminary analysis of crude extracts does not shown some phytochemical in this study also.

In vitro antibacterial assay to assess the efficacy of extract, *S. potatorium* to inhibit the growth of pathogenic microbes showed that the various samples. The minimum inhibitory concentrations of extract, *S. potatorium* was given in Table-2. The results showed that the minimum inhibitory concentration of *S. potatorium* showed the inhibitory effect against all test pathogens at the concentration of 20-40 µg/ml onwards.

The minimal bactericidal concentrations of the extract of *S. potatorium* showed the notable effect on all test pathogens. The minimum bacterial concentration (80-100µg/ml) of the *S. potatorium* showed effect of pathogenic inhibition in comparison to those of streptomycin. *In vitro* antibacterial assay to assess the efficacy of extract to inhibit the growth of pathogenic microbes had broad spectrum of antibacterial potential. Gram positive bacteria and gram negative bacteria like *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris, Klebsiella pneumoniae, Micrococcus* Sp, *Staphylococcus aureus, Citrobacter* Sp and *Serratia marcescens* showed a reduction in their growth on treatment with the extracts. The degree of inhibition as measured by the disc diffusion method, reported that the gram negative bacteria were more inhibited than the gram positive bacteria. Similar findings were also reported by Lighty *et al.,* [7] in extract of red velvet mites and *Aerva lanata* [19]..

IV.CONCLUSION

The present study suggested that using extracts of *S. potatorium* is cheap effective and economic drugs may be prepared for bacterial infections. The active fraction obtained from this *S. potatorium* is an attractive material for leading to possible drug development. The broad spectrum of antibacterial activity of 16- Octadecenoic acid of *S. potatorium* extract is highly promising for further analysis. This fraction can be used as such for

ethanomedicine development with further studies to establish safety and efficacy. Development of ethanomedicines is relatively inexpensive and less time consuming; it is more suitable to our economic conditions compared to allopathic type of drug development.

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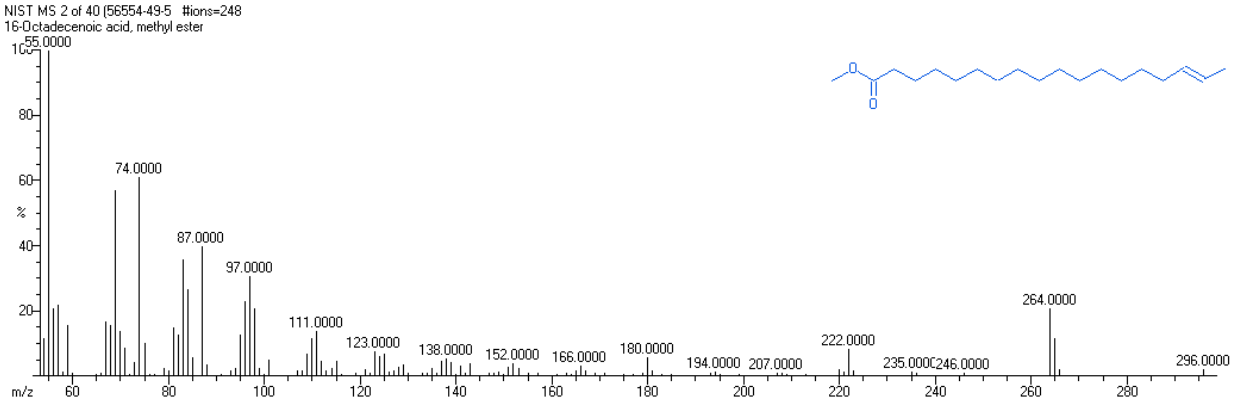

Fig.1. Compound identification of *S. Potatorium* by GCMS method.

Table 1. Antibacterial effect of *S. potatorium* against pathogenic bacteria.

S. No	Bacterial Culture	Zone of inhibition (cm) at different concentration of *S. potatorium* extract (µg/ml)					Streptomycin (µg/ml)	
		20	40	60	80	100	20	40
1	*Escherichia coli*	0.7	0.8	0.9	1.3	1.4	1.2	1.8
2	*Pseudomonas aeruginosa*	0.4	1.2	1.7	1.9	2.1	1.7	2.6
3	*Proteus vulgaris*	0.6	0.8	1.1	1.6	1.9	1.5	2.3
4	*Klebsiella pneumonia*	0.4	0.5	1.5	1.6	1.8	1.6	2.2
5	*Citrobactersp*	0.4	0.6	1`.5	1.6	1.9	1.5	2.2
6	*Serratiamarcescens*	0.2	0.1	0.2	0.1	0.5	0.5	1.0
7	*Micrococcus sp*	0.2	0.8	1.2	1.5	1.7	1.4	2.0
8	*Staphylococcus aureus*	0.3	0.7	1	1.3	1.5	1.2	2.0
9	*Bacillus subtilis*	0.6	0.9	1.2	1.5	1.6	1.4	2.1

Table 2. The minimum bactericidal concentration of *S. potatorium* against pathogenic bacteria.

S. No	Concentration of extracts (µg/ml)	Minimum bactericidal concentration (Optical density value) of extract isolated from *S. potatorium*

		E. coli	*P. aeruginosa*	*P. vulgaris*	*K. pneumonia*	*Citrobactersp*	*S. marcescens*	*Micrococcus sp*	*Staphylococcus aureus*	*Bacillus subtilis*
1	Normal	1.8	1.7	1.9	2.0	2.0	2.0	1.9	1.8	1.9
2	20	1.18	1.46	1.28	1.32	1.42	1.50	1.54	1.62	1.61
3	40	1.16	1.2	1.2	1.2	1.12	1.25	1.5	1.46	1.52
4	60	1.0	1.0	1.0	1.0	1.1	0.8	0.9	0.9	0.46
5	80	0.45	0.56	0.65	0.5	0.56	0.5	0.6	0.72	0.75
6	100	0.18	0.46	0.28	0.32	0.42	0.50	0.54	0.62	0.61
7	Streptomycin – 50	0.34	0.35	0.4	0.42	0.16	0.53	0.52	0.42	0.35
8	Streptomycin – 100	0.16	0.1	0.12	0.13	0.4	0.5	0.25	0.13	0.16

Table 3. The minimum bactericidal concentration of *S. potatorium* against pathogenic bacteria.

S. No	Concentration of extracts (µg/ml)	Minimum bactericidal concentration (Optical density value) of extract isolated from *Catla catla* of Poondireservoir 1								
		E. coli	*P. aeruginosa*	*P. vulgaris*	*K. pneumonia*	*Citrobactersp*	*S. marcescens*	*Micrococcus sp*	*Staphylococcus aureus*	*Bacillus subtilis*
1	Normal	1.8	1.7	1.9	2.0	2.0	2.0	1.9	1.8	1.9
2	20	0.8	0.65	0.85	0.9	0.65	0.9	0.8	0.7	0.85
3	40	0.6	0.5	0.7	0.85	0.6	0.76	0.7	0.65	0.78
4	60	0.4	0.4	0.5	0.62	0.51	0.63	0.53	0.56	0.63
5	80	0.2	0.3	0.4	0.4	0.42	0.41	0.35	0.38	0.41
6	100	0.1	0.2	0.1	0.32	0.2	0.3	0.1	0.2	0.2
7	Streptomycin – 50	0.34	0.35	0.4	0.42	0.16	0.53	0.52	0.42	0.35
8	Streptomycin – 100	0.16	0.1	0.12	0.13	0.4	0.5	0.25	0.13	0.16

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