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PHYTOCHEMICAL SCREENING, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CHLOROFORM AND ETHANOL EXTRACTS FROM *PANICUM TRYPHERON* LEAVES

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ABSTRACT: This study was designed to evaluate the phytochemical findings for the presence of Alkaloids, Flavonoids, Phenols, and Terpenoids, and the phytochemical profile of chloroform and ethanol extracts of *Panicum trypheron* were analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) method. The leaves were dried, powdered, and soaked in chloroform and ethanol, then subjected to GC-MS analysis by using Clarus 680 Gas chromatography. The unknown spectrum was compared with that of the known by using NIST (National Institute of Standards and Technology) database, which revealed the presence of different chemical entities in each of the extracts. The compounds are pharmacologically and biologically noteworthy. The extracts showed good antibacterial activity when tested against selected bacterial strains. This preliminary study gives an overview of the plant, which contains bioactive compounds and is therefore proposed as a pharmaceutically important plant.

INTRODUCTION: Herbal medicines and derivatives prevent the severe effects of modern medical treatments and also play a significant role in preventing and treating many diseases [1]. History has revealed that the plants are chief sources for many proficient and efficient drugs and will remain the chief source for the forthcoming compounds with biological and pharmacological importance [2]. Currently, herbal remedies have become paramount because of their fewer side effects and easy availability [3, 4]. Various health issues in humans are pacified and cured because of the bioactive secondary metabolites present in the medicinal plants [5].

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The crude plant extracts with potential uses and properties are elected through *in-vitro* screening methods, providing the required initial observations. These plant extracts and their properties are used for further pharmacological investigations [6, 7]. These metabolites have an interesting set of biological activities and applications in pharmaceuticals, insecticides, dyes, flavours, fragrances, *etc*. Traditional medicine contains a wide range of substances that can treat many chronic and infectious diseases [8].

Panicum, also called panic grass, is one of the large genera of forage and cereal grasses in the family poaceae, distributed throughout tropical and warm temperate regions of the world. Panic grass could be the response to an uncertain food future. Australian researchers have discovered that a common grass species called panic grass contains enzymes that capture CO_2 from the atmosphere more efficiently than other plants in the extreme

climate conditions predicted in coming decades. *Panicum trypheronschultesmants* **Fig. 1** vernacularly called samai-karuna (tamil) is globally occurring and distributed across China, Indian subcontient and South East Asia, Flowering and fruiting during the months of August-November [9, 10]. It belongs to the family poaceae (Gramineae). It is a common weed around paddy fields in India. The role of *Panicumtrypheron* in the annual recurrence of false smut of rice is reported. *Ustilaginoidea virens* causing false smut in rice was also found to be infecting *P. trypheron*. *P. trypheron* (Ustilagineae) serves as a major source of inoculums [11]. A few grass species in other tribes, for example, *Pennisetum sp*. and *Panicum trypheron* have been reported as hosts of Peronosclerospora [12].

FIG. 1: ***PANICUM TRYPHERON***

11 Small millets and 21 other related wild species of grasses have been identified and collected from southern Rajasthan. During the germplasm collection mission of grasses of Rajasthan, *Panicum trypheron* was reported among them as it grows in moist conditions, grains a scarcity of food was observed.

MATERIALS AND METHODS: The plant was authenticated by a taxonomist (Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai), and the voucher specimen of the plant was preserved in our laboratory, and the voucher number is PARC/2020/4254.

The plant was collected in and around Madanapalle and the whole plant was washed in tap water, rinsed with distilled water, and shade dried for about 15–20 days. The dried leaves were powdered, and a hundred grams of it was subjected to selective sequential extraction using solvents to increase polarity, including Chloroform and Ethanol. The extracts collected were filtered using Whatman filter paper 40, which are then concentrated using a rotary evaporator.

The extracts were analyzed for the presence of Alkaloids, Flavonoids, Phenols and Terpenoids [13] GC-MS analysis and antibacterial activity.

Phytochemical Screening:

Test for Alkaloid: To 2ml of extract, 2ml of concentrated HCl was added. Then few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Test for Flavonoids: To 2ml of extract, 1ml of 2N Sodium hydroxide was added. The appearance of yellow color indicates the presence of flavonoids.

Test for Phenols: 2ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. The formation of blue or green color indicates the presence of phenols.

Test for Terpenoids: The extract (0.5 ml) was treated with 2ml of chloroform and concentrated H_2SO_4. The formation of red-brown color at the interface indicates the presence of terpenoids.

GC-MS Analysis: The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df), and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min.

Acquisition Parameters:

Oven: Initial temp. 60 °C for 2 min, ramp 10 °C / min to 300 °C, holds 6 min,

Total Run Time: 32.00 min.
In auto: 260 °C,
Volume: 1 µl, Split = 10:1, Flow
Rate: 1 ml/min.
Carrier Gas: He.
Column: Elite-5MS (30.0m, 0.25mmID, 250µm df).

Mass Condition (Ei):
Solvent Delay: 2.00 min,
Transfer Temp: 230°C,

Source Temp: 230°C,
Scan: 50 to 600Da,

The parameters used and the conditions were as mentioned above.

Identification of Chemical Constituents: The bioactive compounds obtained from the chloroform and ethanol extracts of *P. trypheron* were recognized based on the Gas Chromatography retention time.

The spectrum of the components was related to the database of known components spectrum present in the NIST library (2008).

Antibacterial Screening: Based on the pharmacological and clinical importance, five strains of bacteria were selected for screening which included *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi and Klebsiella pneumoniae*.

The cultures were obtained from Bharathi Women's College, Chennai, which were grown in nutrient broth at 37°C, and the slants were then prepared on nutrient agar and stored at 4 °C.

The antibacterial activity of chloroform and methanol extracts was studied by well diffusion method [14, 15].

Agar plates were inoculated with 40µl of homogeneous inoculums (1.65 x 10^6 CFU/ml) of each bacterium and were spread using sterile swabs. Wells of 6 mm were made using a sterile borer into agar plates. Five concentrations (50µl, 100µl, 150µl, 200µl, and 250µl) of solvent extracts dissolved in Dimethyl Sulphoxide (DMSO) were transferred into each well.

The positive control used was Ampicillin and DMSO is used as the negative control.

Statistical Analysis: All the experiments were carried out in triplicates. The results are expressed as mean ± standard errors and the comparison of the antibacterial activity of the samples with standard antibiotics was evaluated by applying one-way analysis of variants (ANOVA).

RESULTS AND DISCUSSION:

Analysis of Phytochemicals: The screening for phytochemicals exposed the presence of Alkaloids, Flavonoids, Phenols and Terpenoids in the Chloroform and Ethanol extracts. **Table 1**.

TABLE 1: PHYTOCHEMICAL ANALYSIS

S. no.	Phytochemicals	Chloroform Extract	Ethanol Extract
1	Alkaloids	Present	Present
2	Flavonoids	Absent	Present
3	Phenols	Present	Absent
4	Terpenoids	Absent	Present

Presence of Bioactive Compounds in the Extracts: Bioactive compounds in the chloroform and ethanol extracts are recorded in **Tables 2 and 3**. The compounds were identified and characterized based on the elution order in the Elite-5MS column.

Retention time, percentage area, and the name of the compound and its molecular formula are presented in the table.

The top two compounds based on the richness in the chloroform extract are Heptacosane and Heptadecane, 2, 6, 10, 15-Tetramethyl, whereas, in the ethanol extract, the top compounds are Methyl salicylate and Benzene Butanamine.

TABLE 2: BIOACTIVE COMPOUNDS OF CHLOROFORM EXTRACT OF P.TRYPHERON

Retention Time	Name of the Compound	Molecular Formula	Area%
21.006	Heneicosane, 11-Cyclopentyl	$C_{26}H_{52}$	2.536
21.476	Heptadecane, 2,6,10,15-Tetramethyl-	$C_{21}H_{44}$	6.778
22.006	Heptadecane, 2,6,10,15-Tetramethyl	$C_{21}H_{44}$	12.936
23.126	Heptacosane	$C_{27}H_{56}$	14.691
24.292	Tetratetracontane	$C_{44}H_{90}$	9.173
24.882	Hentriacontane	$C_{31}H_{64}$	6.682
25.407	Heptacosane, 1-Chloro-	$C_{27}H_{55}Cl$	4.628
26.463	Tritetracontane	$C_{43}H_{88}$	2.171
27.013	14-Heptadecenal	$C_{17}H_{32}O$	2.652
27.468	2,4-Cyclohexadien-1-One, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy	$C_{14}H_{22}O_2$	4.265
28.889	URS-12-EN-28-OL	$C_{30}H_{50}O$	4.036

TABLE 3: BIOACTIVE COMPOUNDS OF ETHANOL EXTRACT OF *P. TRYPHERON*

Retention Time	Name of the Compound	Molecular Formula	Area%
16.0204	Methyl salicylate	$C_8H_8O_3$	29.852
16.509	Benzoic Acid, 2-(Acetyloxy)-, Methyl Ester	$C_{10}H_{10}O_4$	10.904
18.885	Benzenebutanamine	$C_{10}H_{15}N$	20.232
27.043	1,3-Bis-T-Butylperoxy-Phthalan	$C_{16}H_{24}O_5$	2.655
27.308	4-Hydroxy-.Beta.-Ionone	$C_{13}H_{20}O_2$	16.785
27.683	Pseduosarsasapogenin-5,20-Dien Methyl Ether	$C_{28}H_{44}O_3$	10.104

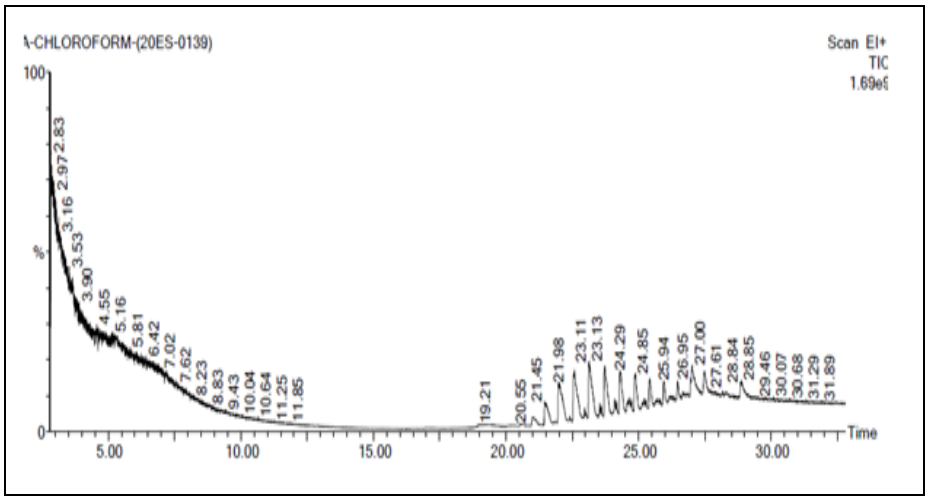

FIG. 2: CHLOROFORM EXTRACT GAS CHROMATOGRAM

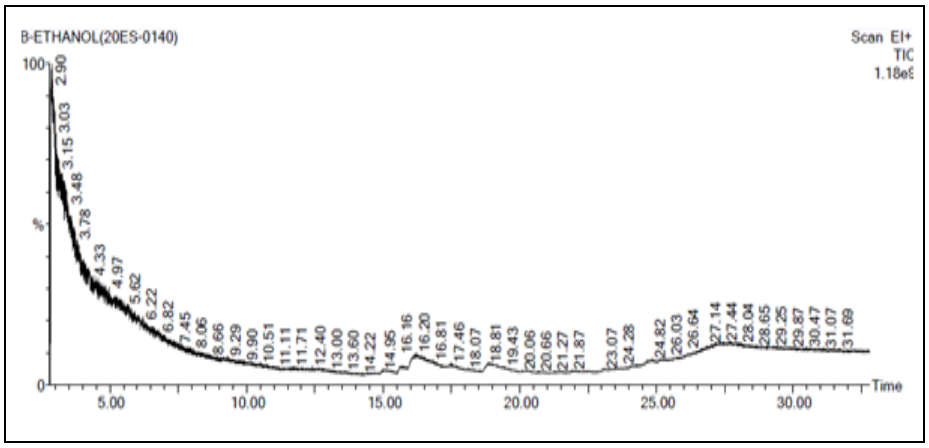

FIG. 3: ETHANOL EXTRACT GAS CHROMATOGRAM

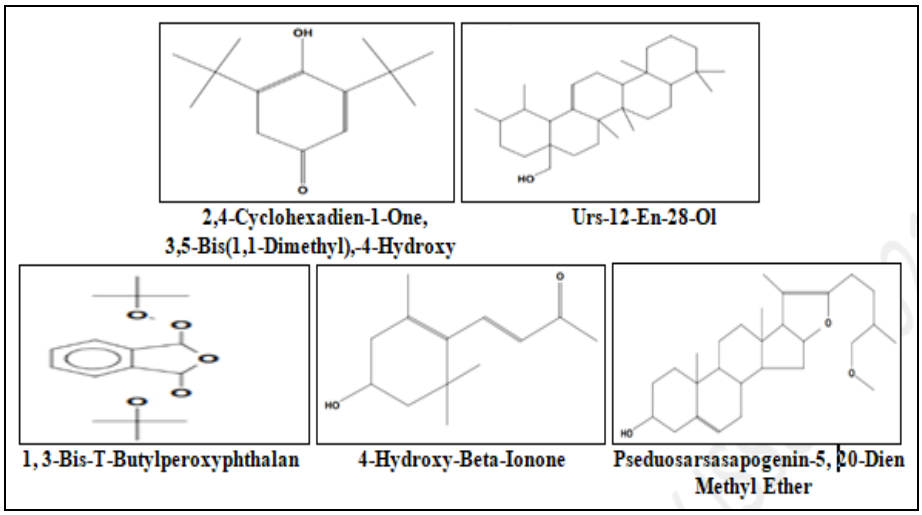

FIG. 4: STRUCTURES OF SOME OF THE COMPOUNDS SCRUTINIZED BY GC-MS

The GC-MS analysis revealed many compounds which are bioactive from both the chloroform and ethanol extracts. The two extracts had no common compound in them. The compounds analyzed have many pharmacological activities which aid the plant in self-healing. The compound methyl salicylate releases Musculo skeleton pain in the muscles, joints and tendons. It is pharmacologically similar to Aspirin and other NSAIDS but as a topical agent it primarily acts as a rubefacient and skin irritant [16]. The Heptacosane present in the chloroform extract has multiple biological and pharmacological activities, which include anti-oxidant, anti-inflammatory, antimicrobial, anti-cancer and antiallergic [17].

Antibacterial Activity: The antibacterial activity of different extracts of the plant *P. trypheron* was done by well diffusion method with DMSO as negative control and Ampicillin as a positive control. The measured zone of inhibition was recorded **in Tables 4 & 5**. The two extracts showed good antibacterial activity.

TABLE 4: ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF *P. TRYPHERON*

Pathogen	Concentration and zone of inhibition					Ampicillin 5mg/ml
	50 µl	100 µl	150 µl	200 µl	250 µl	
Escherichia Coli	3.1 ± 0.2	5.±0.15	7.1±0.2	10.3±0.3	13.1±0.3	14
Pseudomonas aeruginosa	3.8±0.4	6.1±0.15	8.4±0.1	11.6±0.15	14.2±0.4	14
Salmonella typhi	4.4±0.4	5.2±0.15	10.4±0.17	13.5±0.25	15.4±0.2	14
Salmonella paratyphi	4±0.37	6±0.1	10.7±0.15	13.8±0.15	14.9±0.15	14
Klebsiella pneumonia	3.5±0.35	6.3±0.15	7.7±0.15	9.7±0.2	11.5±0.3	14

Data given are mean of three values ± Standard error.

TABLE 5: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *P. TRYPHERON*

Pathogen	Concentration and zone of inhibition					Ampicillin 5mg/ml
	50 µl	100 µl	150 µl	200 µl	250 µl	
Escherichia Coli	-	-	5.2±0.41	8±0.11	9.9±0.3	16
Pseudomonas aeruginosa	-	-	6.1±0.2	7.5±0.35	9.5±0.05	16
Salmonella typhi	-	-	5.4±0.35	8.4±0.2	11.2±0.15	16
Salmonella paratyphi	-	-	6.3±0.15	8.7±0.2	10.9±0.1	16
Klebsiella pneumonia	-	-	6.1±0.1	9.6±0.2	14.2±0.3	16

Data given are mean of three values ± Standard error.

The results showed that the two extracts are hypothetically effective against the microbes. The chloroform extract is effective from a minimum concentration of 50µl, whereas ethanol extract was effective from 150µl against tested bacteria. The antibacterial activity increased linearly with the increase in concentration.

CONCLUSION: The extracts from the plants were used as traditional medicines overages, and about 80% of the world's population depends on traditional therapies. The present work reveals the medicinal applications of the plant, which are supported by biological activities. From this, it is concluded that the plant P. trypheron exhibited antibacterial activity that is defensible by bioactive compounds analyzed through GC-MS analysis. The compounds isolated by GC-MS analysis explained the correlation between the biological activity and phytochemical constituents. The present study justifies the uses of this plant in the traditional system of medicine for treating infectious diseases caused by microbes. Additional purification and structure elucidation of the compounds increases the health benefits from the plant prominent to further biological and pharmacological studies along with clinical trials.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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