Research Article

Chemical Profiling and *in vitro* Cytotoxic Properties of *Themeda triandra* Forssk. Against Human Bone Osteosarcoma Cell Line (MG-63)

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ABSTRACT

Aim: Grasses are an important group of monocotyledonous plants that are utilized as healthy foods as well as remedies against diseases for both humans and livestock. *Themeda triandra* Forssk. is a grass, which belongs to the family Poaceae. It was collected from the forest and grassland regions of Chikkamagaluru District, Karnataka. The goal of the present investigation is to identify the metabolites and in vitro cytotoxic properties against Osteosarcoma cancer (MG-63) cell lines. **Materials and Methods:** The phytochemical investigations were done by preliminary qualitative and quantitative GCMS analysis. In vitro cytotoxic potential against MG-63 cells was examined by MTT assay. **Results:** Qualitative phytochemical investigations showed the existence of various metabolites such as alkaloids, phenols, tannins, flavonoids, glycosides, steroids, terpenoids, proteins and carbohydrates. GCMS profiling of methanolic whole plant extract exhibited the occurrence of 5 phytocompounds, with valuable pharmacological properties. The whole plant different solvent extracts showed moderate cytotoxicity against MG-63 cell lines at higher concentrations. Among the different solvent extracts petroleum ether extracts showed significant potential with an IC_{50} value of 28.82 µg/mL. **Conclusion**: It was concluded that *Themeda triandra* consists of several phytoconstituents and is also a proven effective cytotoxic agent for MG-63 cell lines, further investigations need to determine the molecular mechanism behind cytotoxic potential and elucidation of the potential pharmacological properties required further investigation

Keywords: *Themeda triandra*, Phytochemicals, GCMS analysis, Osteosarcoma cancer (MG-63), MTT assay.

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INTRODUCTION

Cancer is distinguished by abnormal cell proliferation and expansion. a multi-phase process that includes the transition of a precancerous lesion into a state of malignant tumour state is how cancer begins: normal cells are transformed into tumor cells. These changes arise from the interaction of physical factors such as ultraviolet radiation, chemical factors like tobacco and asbestos and biological factors like viruses and bacteria with an individual's inherited features.[1] Cancer ranks among the top causes of death and a key impediment to increasing people's life expectancy in every country on the earth. According to the Global Cancer Observatory (GLOBOCAN), Globally, 19.3 million more cases of cancer will be diagnosed in 2020.[2] After China and the United States, India came in third. GLOBOCAN predicted that the number of cancer cases in India would rise to 2.08 million by 2040, a 57.5% increase from 2020.[3]

Osteosarcoma, which is the most common major bone tumor, primarily affects children and young adults.[4]

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Currently, between 50-65% of patients survive overall when treated with a strict chemotherapy regimen both before and after surgery.[5] This is a significant condition since bones are thought to be high-risk areas for metastasis of other malignancies. However, information on bone malignancies and the availability of medicines for such cancers are insufficient.[6]

Chemotherapeutic synthetic substances are intended to target cancer cells; however, they may also destroy healthy tissues which leads considerable reduction in patient anticipated survival. As a result, therapeutic drugs that are new, target-centered, safer and low-cost are necessary. Medicinal herbs are both affordable and secure. As a result, they may be a viable option for novel anticancer drugs.[7]

Themeda triandra Forssk. is a common widespread grass species in India, which is a family member of Poaceae. It is often known as kangaroo grass in Australia.[8] It has been traditionally used to cure wounds[9] and cure malaria.[10] There is very little pharmacological significance for the bioactive compounds found in plant species, despite their abundance. However, in recent years Kangaroo grass has been looked upon as a weed that is eaten by livestock and the value of medicine in various diseases like cancer is not much known. Hence, the present investigations focused on phytochemical profiling and in vitro cytotoxic properties of whole plant methanolic extracts of *Themeda triandra* Forssk. against Human Bone Osteosarcoma Cell Line (MG-63)

MATERIALS AND METHODS

Plant samples collection

The whole plant of *Themeda triandra* is collected from the roadsides and railway lines of Ramanahalli, which is located in the district of Chikkamagaluru, Karnataka, India. The obtained plant samples were taxonomically identified by using Gamble flora (Gamble, 1935) and confirmed by CARI (Central Ayurveda Research Institute) and a voucher sample was placed in the herbarium, CARI Bangalore with specimen number (RRCBI-mus 432).

Preparation of the whole plant extracts

The collected plants were washed with water to remove soil matter and kept for shade drying for about 5-6 days. After that, the dried whole plant samples were pulverized with the help of a mechanical pulverizer. About 250 g of whole Plant samples were soxhlet extracted with petroleum ether, methanol and distilled water. After extraction, the extracts were refrigerated for later use.

Qualitative screening phytoconstituents

The extracted extracts were stored in different crude extracts of whole plant samples were screened for preliminary metabolites which includes Alkaloids, Tannins, Glycosides, Phenols, Sterols, Flavonoids, Saponins, Terpinoids, Proteins and Carbohydrates by using standard protocols.[11,12]

GCMS profiling

The mass spectrophotometer and Agilent 19091-433HP, USA, 7890A gas chromatograph system were utilized for the GC-MS analysis. The system was fitted with a 5675C Inert MSD with a Triple-Axis detection device, coupled with an HP-5 MS fused silica column (5% phenyl methyl siloxane, 30.0 m × 250 µm, film thickness 0.25 µm). The carrier gas used in this experiment was helium, which had a column velocity of about 1.0 mL/min.

The additional GC-MS parameters included a 250ºC ion source temperature, a 300ºC interface temperature, 16.2 psi pressure, 1.8 mm time and a 1 µL split mode injector with a 1:50 split ratio and 300ºC injected temperature. After starting at 36ºC for 5 min, the column temperature rose at a rate of 4ºC/min to 150ºV. The temperature was increased to 250ºC at a rate of 20ºC/min and held there for 5 min. The elution took 47.5 min in total. Through the comparison of each component's average peak area to the total area; we were able to calculate the relative percent amount of each component. The entire system was managed and data was gathered using software provided by the supplier, MS Solution.

Identification of constituents

The NIST database was used to analyze the mass spectra and identify the components based on their retention indices. More than 62,000 patterns of known chemicals are included in the database. The spectra of the unknown constituents were compared to the standard mass spectra of the known constituents kept in the NIST collection.

MTT cell viability assay

The cytotoxic effect of the whole plant extract was assessed by MTT cell cytotoxicity assay against the MG-63 cell line (Cell line was purchased from NCCS, Pune, India). The working solution (1% v/v) was made by dissolving the extract in DMEM. The desired cell density of 20,000 cells per well, or 200 µL of cell suspension, was added to a 96-well plate and left overnight to incubate without the test agent. The cell suspension-containing microtiter plate was

incubated for 24 hr at 37°C with 5% CO_2 and various amounts of whole plant methanolic extract (12.5 µg mL^{-1}, 25 µg mL^{-1}, 50 µg mL^{-1}, 100 µg mL^{-1} and 200 µg mL^{-1}) added. The positive control in this study was doxorubicin, whereas the negative control was DMSO. Following the incubation period, each well received 100 µL of DMSO solubilization solution and the MTT reagent. Tecan Infinite, Austria provided an ELISA plate reader that was used to record the absorbance of the reaction mixture in every well at 570 nm. The linear regression equation was utilized to ascertain the IC_{50} values. i.e. Y = Mx + C here y = 50, M&C values are produced from the viability graph.

RESULTS

Qualitative screening phytoconstituents

Qualitative screening of metabolites yielded results indicating the presence of different phytochemicals, including alkaloids, tannins, steroids, phenols, flavonoids, glycosides, terpenoids, proteins and carbohydrates

Table 1: Qualitative analysis of different solvent extracts of *Themeda triandra*.

Secondary metabolites	Assay names	Petroleum ether	Methanol	Aqueous
Alkaloids	Mayer's assay	+	+	-
	Wagner's assay	+	+	+
	Dragend-roff's assay	+	+	+
Tannins	Ferric chloride assay	+	+	+
	Lead acetate assay	+	+	+
	Salkowski's assay	–	+	–
	Gelatin assay	–	–	–
Glycosides	Salkowski's test	+	–	–
	Keller-killani's assay	+	–	–
Phenols	Ferric chloride assay	–	+	+
Flavonoids	Ferric chloride assay	+	+	+
	Lead acetate assay	+	+	+
	Shinoda assay	–	+	–
Sterols	Libermann Burchard's assay	–	+	–
Saponins	Foam assay	–	+	+
Terpinoids	Libermann Burchard's assay	–	+	–
Carbohydrates	Benedict's assay	+	+	+
	Fehling's assay	–	+	+
Proteins	Biuret assay	–	+	+
	Millon's assay	+	–	–
	Ninhydrin assay	–	–	–

GCMS profiling

The chromatogram of GCMS profiling of whole plant methanolic extracts of *Themeda triandra* showed the occurrence of five peaks indicating the existence of five phytoconstituents (Figure 1). The identified phytochemicals along with their retention time, peak area %, Molecular Formula (MF), Molecular Weight (MW) and biological activities were listed in the table (Table 2).

The results showed that, Bis(2-ethylhexyl) phthalate was found to be a major compound with a peak area of 39.36% followed by Pterin-6-carboxylic acid with a peak area of 35.45%; Tetraacetyl-d-xylonic nitrile with a peak area of 18.65% and Cycloserine with a peak area of 4.70% and least peak was showed by Benzenemethanamine, N-methyl-with a peak area of 1.83% in the whole plant methanolic extracts of *Themeda triandra*

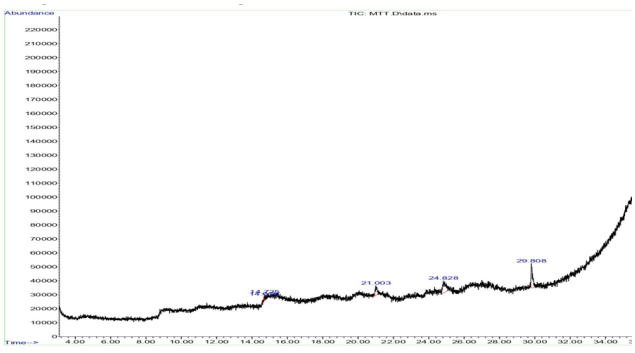

Figure 1: GCMS chromatogram of whole plant methanolic extracts of *Themeda triandra*

Table 2: List of identified phytocompounds in crude whole plant methanolic extracts of *Themeda triandra* by GC-MS analysis

RT	Peak area %	Chemical compound present	Molecular formula	Molecular weight	Properties
14.679	4.70	Cycloserine	$C_3H_6N_2O_2$	102.09	Antituberculosis, cytotoxic and antimicrobial properties.
14.725	1.83	Benzene-methanamine, N-methyl-	$C_8H_{11}N$	121.17	Antibacterial activity.
21.003	18.65	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343.29	Antiviral and antioxidant activities.
24.828	35.45	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207.15	Antioxidant and anti-inflammatory activity and also used in folic acid Estimation.
29.808	39.36	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.60	Antimutagenic, antibacterial, larvicidal and cytotoxic activities.

Effect of whole plant different solvent extracts of *Themeda triandra* on MG-63 cell lines.

In vitro cytotoxicity of whole plant different solvent extracts of *Themeda triandra* was evaluated against Human Bone Osteosarcoma Cell Line (MG-63) using MTT assay at different dosages (12.5, 25, 50, 100 and 200 µg/mL) and 24 hr of the incubation period. The MTT assay results revealed that, whole plant different solvent extracts of *Themeda triandra* exhibited concentration-dependent cytotoxicity against Human Bone Osteosarcoma cells (MG-63) (Figure 2). The whole plant different solvent extract showed moderate cytotoxicity against MG-63 cell lines at higher concentrations when compared with the standard Doxorubicin, which is a commonly used medication to treat Human Bone Osteosarcoma cancer. Among the different solvent whole plant extracts of *Themeda triandra*, petroleum ether extract exhibited the maximum activity followed by methanolic extract and aqueous extract. The minimum inhibitory concentration IC_{50} value of whole plant petroleum ether extracts was 28.82 µg/mL, followed by the methanolic extract showed an IC_{50} value of 41.36 µg/mL and the aqueous extract showed an IC_{50} value of 125.24 µg/mL (Figure 3).

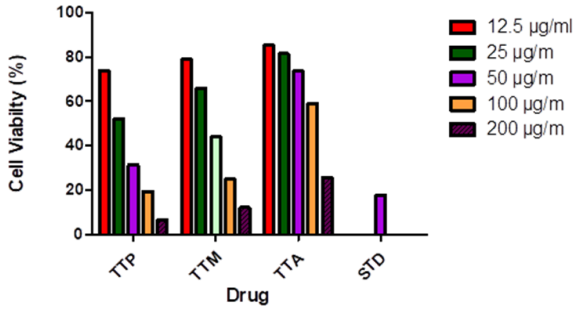

Figure 2: Antiproliferative effect of *Themeda triandra* different solvent extract of Petroleum ether (TTP), Methanol (TTM) and Aqueous (TTA) against MG -63 cell line on 48 hr treatment, doxorubicin (5 µg/mL) used as a positive control.

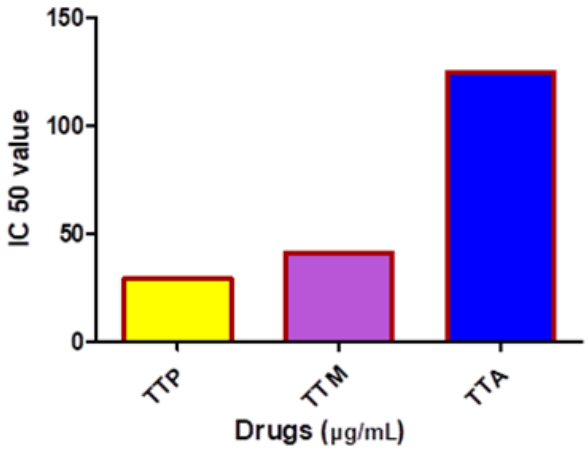

Figure 3:Cytotoxicity activity of *Themeda triandra* different solvent extracts of Petroleum ether (TTP), Methanol (TTM) and Aqueous (TTA) against MG -63 cell lines on 48 hr (MTT assay; mean IC_{50} values).

DISCUSSION

The results of the preliminary qualitative investigations of phytochemicals revealed that the *Themeda triandra* exhibited the presence of various phytoconstituents (Table 1). Numerous pharmacological activities of these phytoconstituents include antibacterial, anti-inflammatory, hepatoprotective, anti-allergic, anti-cancer, anti-diabetic, insecticidal, anti-malarial and antiviral effects[13] The primary bioactive components are phenols, which also serve as free radical scavengers, immune system stimulators, gene expression regulators and antibacterial agents.[14] Tannins were previously reported to have anti-inflammatory, cardiovascular-protective and antibacterial effects.[15,16] According to Wang *et al.*, flavonoids were used as diabetic, antibacterial, anti-inflammatory and anti-aging drugs.[17] Further saponins have hypotensive, cardiac depressing, cardiotonic, hemolytic and other

functions.[18] It has been previously reported cure wounds and malaria,[9-10] these activities may be due to the presence of these bioactive compounds either singly or combination effect.

Regularly, phytochemical investigations are being developed and precisely certified. GCMS is an effective tool for the identification of plant bioactive compounds.[19] The GCMS analysis of the whole plant methanolic extract of *Themeda triandra* showed five phytoconstituents, all the identified phytoconstituents have valuable therapeutic properties (Table 2 and Figure 1). It is the first report of the GCMS analysis of *Themeda triandra*. The identified compound Cycloserine reported to exhibit antituberculosis, cytotoxic and anti-microbial properties.[19-21] Further Benzenemethanamine, N-methyl- possesses antibacterial properties.[22] The compound Tetraacetyl-d-xylonic nitrile has been shown to have a variety of biological functions, including antiviral and antioxidant activities.[23,24] Pterin-6-carboxylic acid has antioxidant and anti-inflammatory properties and is also used in folic acid estimation.[25,26] Bis(2-ethylhexyl) phthalate is a major identified phytoconstituent that has antimutagenic, antibacterial, larvicidal and cytotoxic properties.[27-30] The presence of these compounds might be a reason behind all the ethnopharmacological properties of *Themeda triandra*.

The whole plant different solvent extracts showed moderate cytotoxicity against MG-63 cell lines (Figure 2). Several grass species belonging to the Poaceae family have been reported to have cytotoxic properties against several cancer cells.[31-34] In the present investigations various extracts of *Themeda triandra*, have been reported that exhibit significant and dose-dependent cytotoxic action against, MG-63 cell lines. Similar dose and time-dependent cytotoxic activities were observed in various grass species including *Triticum aestivum*, against Oral Cancer (KB) Cells, *Lophaterum gracile* against breast cancer cells T47D, *Cymbopogon citratus* against VERO (kidney cells) and SiHa cell lines (cervical cancer cells) and *Cymbopogon flexuosus* against Ehrlich Ascites Carcinoma (EAC) cells.[35-38] It was the first report on the cytotoxic activities of *Themeda triandra* against MG-63 cell lines. The MTT experiment indicated that, the majority of the chemicals identified in GC-MS analysis such as, Bis (2-ethylhexyl) phthalate and Cycloserine reported anticancer properties. The compounds Pterin-6-carboxylic acid and Tetraacetyl-d-xylonic nitrile were to possess antioxidant properties (Table 2). Whereas, the compounds with antioxidant properties also exhibited cytotoxic effects by preventing the growth of numerous cancer cells.[39]

The outcomes demonstrated that, among the different solvent whole plant extracts of *Themeda triandra*, petroleum ether extract exhibited the maximum activity followed by methanolic extract and aqueous extract. This cytotoxic properties may be due to the synergetic effect of the different compounds present in the whole plant extract. As a result, whole plant extracts may inhibit cell proliferation due to several bioactive phytoconstituents, phenolic compounds and other anti-oxidants found in *Themeda triandra*.[40]

CONCLUSION

It was concluded that, the grass *Themeda triandra* has various phytoconstituents. GCMS profiling of methanolic whole plant extract revealed the occurrence of 5 phytocompounds, with valuable therapeutic uses. Among Bis (2-ethylhexyl) phthalate was the major identified compound. The whole plant's different solvent extracts showed moderate cytotoxicity against MG-63 cell lines at higher concentrations. Further investigations are needed to be conducted to know the molecular mechanism behind cytotoxic potential. Consequently, the benefits of *T. triandra* extract as a possible source of herbal medications and as a dietary supplement for people and animals are being analyzed and quantified in further research. However, for these plant species to find successful uses in the biomedical and pharmaceutical industries, more thorough research is required to fully understand their extensive nutritional qualities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MTT: 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; **GCMS:** Gas Chromatography-Mass Spectroscopy Analysis; **MG-63:** Human Bone Osteosarcoma Cell Line; **NCCS:** National Centre for Cell Science; **hr:** Hour; **min:** Minute; **NIST:** National Institute Standard And Technology; **IC_{50}:** Inhibitory concentration at 50% growth; **µg:** Microgram; **mL:** Milliliter; **nm:** Nanometer.

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