Research Article

Preliminary Phytochemical, Gas Chromatography-Mass Spectroscopy Analysis and *in-vitro* Anticancer Activities of *Macrosolen parasiticus* (L.) Danser on Human Prostate Cancer Cell line (PC-3)

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ABSTRACT

Background: *Macrosolen parasiticus* (L.) Danser, a hemiparasitic mistletoe plant was collected from the Bhadra Wildlife Sanctuary to determine its phytoconstituents and *in vitro* anticancer activities against PC- 3 cancer cell lines. **Materials and Methods:** The collected samples were subjected to a hot extraction by using a soxhlet extractor. Investigation of phytochemicals was done by preliminary qualitative and GC-MS screening. *In vitro* anticancer activities on PC-3 cell lines were determined by MTT assay. **Results:** Preliminary phytochemical analysis revealed the occurrence of a variety of phytochemicals. GCMS screening of leaf methanol extract showed the occurrence of sixteen phytoconstituents, with valuable therapeutic uses. The identified major phytoconstituents were Dihydrochrysin (45.03%); 1,6-Anhydro-beta-D-glucopyranose (15.42%), and minor constituents were Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]- (0.95%); 4alpha-Phorbol 12,13-didecanoate (0.84%) and Agaricic acid (0.49%). The methanolic leaf extract showed moderate cytotoxicity on PC-3 lines at higher concentrations with IC_{50} values of 448.7µg/mL. while it had no cytotoxic effects on MEF-L929 non-cancerous cells. **Conclusion:** It is concluded that *Macrosolen parasiticus* contains a wide variety of secondary metabolites and also it proved a potential anticancer agent for PC- 3 cells. Therefore, more research is required to determine its biological activities.

Keywords: *Macrosolen parasiticus,* Mistletoe, Phytochemicals, GC-MS analysis, Prostate cancer (PC-3).

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INTRODUCTION

Prostate cancer is one of the most frequent cancers in men, especially in industrialized nations, where the high prevalence is recognized in males over 65.[1] Surgery, radiation, and hormonal therapy are the most common treatments for prostate cancer, but they all have major side effects, such as urinary incontinence and sexual dysfunction.[2] Dietary supplements including vitamins, soy proteins, carotenoids, and herbal tea have all been used to treat prostate cancer.[3-5] According to the WHO, roughly 65% of the global population prefers to cure sickness with herbal and traditional treatments.[6] Herbal medicine and natural sources contribute for about approximately 60% of anticancer medicines; however, there are still several plants with anticancer potential that have not yet been completely explored. As a result of the adverse effects of pharmaceutical drugs, the use of complement natural remedies is an alternative solution, as there have been few studies on the usage of medicinal herbs in treating prostate cancer.[7]

Plants' therapeutic value is derived from natural sources of phytochemical elements that have a beneficial physiological effect on humans.[8] Flavonoids, alkaloids, and terpenes are phytochemicals that have gained a lot

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of interest in current centuries because of their varied biological activities, particularly cancer chemopreventive and cytotoxic actions.[9] Plants generate secondary metabolites, which have a wide range of molecular structures and properties and are of tremendous medicinal value.[10] Plant-produced secondary metabolites are of increasing scientific interest, due to the rapid emergence of resistance to routinely utilized drugs, which has resulted in a serious public health concern and a worldwide problem, resulting in a major problem for mankind.[11]

Macrosolen parasiticus (L.) Danser a hemiparasitic mistletoe plant that belongs to the Loranthaceae family used in ethnoveterinary remedies and leaf paste used to eliminate ticks.[12] It grows abundantly in the Western Ghats region, on *Ficus religiosa*, *Mangifera indica, Azadirachta Indica,* and *Artocarpus heterophyllus*.[13] It has been previously reported to have antioxidant activity[14] and anticancer activity against Ehrlich's ascites carcinoma and MCF- 7 cell lines.[15-16] However, the anti-cancer properties of this plant have not been investigated on prostate cancer cell lines(PC-3). Thus, we aimed to test the efficiency of this medicinal plant on PC-3 cell lines. This study aimed to examine phytoconstituents and *in vitro* anticancer activity on prostate cancer cell lines (PC-3).

MATERIALS AND METHODS

Plant Materials Collection

The leaf samples of *Macrosolen parasiticus* (L.) Danser was collected from the Bhadra Wildlife Sanctuary. The botanical identification of plant was done with the help of the flora (Gamble, 1935) and a voucher sample is placed in the herbarium, Department of PG Studies and research in Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga, with a voucher specimen number (KU/AB/RN/KPS-01).

Preparation of the Extract

The collected leaf samples were cleaned with the help of water, after about 20- 25 days of drying in the shade, and mechanically powdered. About 750 g of powdered material (leaves) was subjected to Soxhlet extraction by using petroleum ether, chloroform, and methanol. Air-dried and stored in airtight bottles.

Qualitative Tests for Phytochemicals

The crude extracts of plant samples were subjected to phytochemical analysis for the detection of different secondary metabolites such as tannins, alkaloids, saponins, flavonoids, phenols, steroids, glycosides, and carbohydrates using standard methods.[17-18]

GCMS Investigation

GCMS screening of the methanolic leaves extract of *M. parasiticus* was carried out with the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The apparatus has a DB 35 – MS Capillary Standard non-polar column with 30 mm × 0.25 mm ID × 0.25 µm film dimensions. Helium is employed as the carrier gas, with a flow rate of 1.0 ml/min flow detector was set to 250°C, and the oven was set to the following temperature: After 15 min at 60°C, the temperature was steadily increased to 280°C for 3 min. identification of phytoconstituents was based on the interpretation of the mass spectrum of samples by using NIST, the unidentified constituents were matched with the spectra of the identified constituents deposited in the NIST collection.

Cell Lines Culture

The PC-3 cell lines were obtained from the NCCS in Pune. The cells were subcultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% nonessential amino acids in a culture flask, and incubated in a 5% CO_2 and 95% atmospheric humidity. The viability of the cells was determined using a hemocytometer after trypsinization. An MTT experiment was carried out with 20,000 cells per well in 200µl cell suspension plated into 96-well plates.

Test Groups

M. parasiticus leaf methanolic extract was used to treat PC-3 cell lines. The desired amounts of test chemicals were produced in DMSO before a test. The reactant solutions were mixed with media, and cells were treated with varying doses (31.25 to 500µg/mL) of the extract and incubated for 24 hr. The effects of induced were compared to those of regular medication, curcumin. The trial will be divided into various treatments. – ve control: cell lines only. + ve control: cell lines + curcumin (10µM). Treatment group: cell lines + methanolic extracts. For the normal MEF-L239 cell line, the same treatments were used.

MTT Cell Viability Assay

Remove the plates from the incubators after 24 hr, discard the wasted media, and add the MTT reagents to a maximum dose of 0.5mg/mL of the overall amount. Return plates to an incubator for a 3hr incubation period. Following incubation, the formazan generated was dissolved with a 100µL DMSO solution. After

shaking the suspension for 5 min, an ELISA reader was used to measure the absorbance at 540nm and 630nm. The inhibitory concentration at 50% growth (IC_{50}) was determined.

RESULTS

The Compound Yield of Plant Extracts

The quantity of yield of extracts from *M. parasiticus* leaves by using petroleum ether, chloroform, and methanol were 15.32 g, 23.8 g, and 65.35g (weight), respectively, concerning the shade dried samples.

Phytochemicals Screening

Qualitative phytochemical analysis of *M. parasiticus* reveals the existence of a variety of secondary metabolites. The occurrence of alkaloids, tannins, glycosides, phenols, flavonoids, saponins, terpenoids, and carbohydrates was identified in methanolic extract. Sterols were identified in petroleum ether and chloroform extracts and glycosides were identified in all three solvents (Table 1).

Table 1: Preliminary qualitative phytochemical screening of *M. parasiticus* leaf extracts.

Secondary metabolites	Name of the test	Petroleum ether	Chloroform	Methanol
Alkaloids	Mayer's test	-	-	+
	Wagner's test	-	-	+
	Dragendorff's test	-	-	+
	Tannic acid test	-	-	+
Tannins	Ferric chloride test	-	-	+
	Gelatin test	-	-	+
	Lead acetate	-	-	-
	Salkowski's Test	-	-	+
Glycosides	Keller-killiani's test	+	+	+
	Legal's test	+	+	+
	Salkowski's Test	+	+	+
Phenols	Ferric chloride test	-	-	+
Flavonoids	Ferric chloride test	-	-	+
	Shinoda test	-	-	+
	Alkaline reagent test	-	-	+
	Lead acetate test	-	-	+
Sterols	Libermann Burchard's Test	+	+	-
Saponins	Foam test	-	-	+
Terpenoids	Libermann Burchard's Test	-	-	+
Carbohydrates	Benedict's Test	-	-	+
	Fehling's Test	-	-	+

-: Negative result; +: Positive results

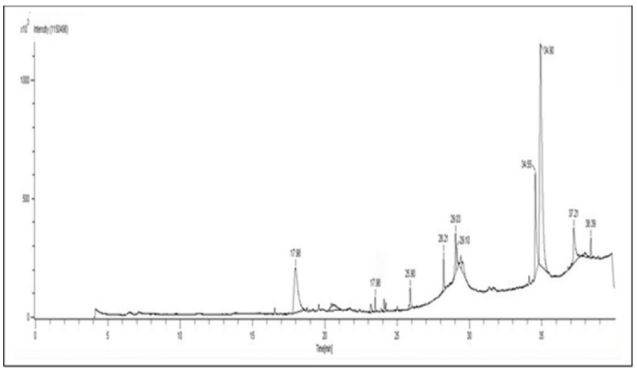

Figure 1: GC-MS chromatogram of methanolic leaf extract of *M. parasiticus* (L.) Danser.

GCMS analysis of Leaf Methanolic Crude Extract

GC-MS chromatogram of *M. parasiticus* methanolic leaf extract shows the presence of sixteen peaks indicating the presence of sixteen bioactive phytochemical compounds (Figure 1). The identified phytoconstituents along with therapeutic uses were listed in Table 2.

The result revealed that, Dihydrochrysin (45.03%) was found as major component followed by 1,6-Anhydro-beta-D-glucopyranose (15.42%); Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester (11.60%); 9-Hexadecenoic acid (6.21%); Oleic acid (3.82%); (5beta)Pregnane-3,20beta-diol,14alpha,18alpha-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,diacetate (2.78%); 11-Octadecenoic acid, methyl ester (2.48%); Octadeca-9,12-dienoic acid (2.10%); Glyceryl 1,2-dipalmitate (2.04%); 1,2,3,4,5-Cyclopentanepentol (1.99%); n-Hexadecanoic acid (1.82%); d-Mannose (1.20%); E-3-Pentadecen-2-ol (1.15%); Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-(0.95%); 4alpha-Phorbol 12,13-didecanoate (0.84%) and Agaricic acid (0.49%) in the methanolic leaf extract of *M. parasiticus*.

Effect of *M. parasiticus* Leaf methanolic extracts on PC-3 cell lines

The results of the MTT assay showed that the proportion of inhibitory activity by *Macrosolen parasiticus* methanolic leaf extract at concerning concentrations from 31.2 to 500 µg/mL was determined after 24 hr (Figure 2). Methanolic leaf extract showed moderate anticancer effects on PC-3 cell lines at higher concentrations and this extract showed no cytotoxic effects on MEF-L929 non-cancerous cell lines using the MTT assay. At higher concentrations, the extracts have an anticancer effect comparable to that of established chemotherapeutic medications like curcumin 10µM, it is generally used to treat cancer. The IC_{50} values of methanol extract of *M. parasiticus* against, PC-3 cell lines are 448.7µg/mL (Table 3).

Table 2: List of identified Phyto-compounds in crude leaf methanolic extract of *M. parasiticus* (L.) Danser by GC-MS analysis.

Retention time (RT)	Average Percentage	Chemical compound present	Molecular formula	Molecular weight	Therapeutic uses
17.98	15.42	1,6-Anhydro-beta-D-glucopyranose	$C_6H_{10}O_5$	162	No significant report.
20.45	2.78	(5beta)Pregnane-3,20beta-diol, 14alpha,18alpha-[4-methyl-3-oxo- (1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	489	No significant report.
23.18	0.95	Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-	$C_{20}H_{22}N_6O_6$	442	No significant report.
23.48	1.15	E-3-Pentadecen-2-ol	$C_{15}H_{30}O$	226	No significant report.
24.09	0.84	4alpha-Phorbol 12,13-didecanoate	$C_{40}H_{64}O_8$	672	Antiviral activity.[31]
24.22	0.49	Agaricic acid	$C_{22}H_{40}O_7$	416	Antimicrobial and cytotoxic activity.[33]
25.90	2.04	Glyceryl 1,2-dipalmitate	$C_{35}H_{68}O_5$	568	Antimicrobial activity.[32]
28.21	2.48	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Antibacterial activity. [34]
29.03	3.82	Oleic acid	$C_{18}H_{34}O_2$	282	Antifungal, antioxidant, apoptotic activity.[29-30]
29.10	1.82	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, hypocholesterolemic, nematicidal, pesticide, hemolytic, antiandrogenic, hemolytic, 5-alpha reductase inhibitor, and anti-Inflammatory activity.[27-28]
29.40	1.20	d-Mannose	$C_6H_{12}O_6$	180	Immunostimulatory, anti-tumor, antibacterial activity.[37-38]
29.47	1.99	1,2,3,4,5-Cyclopentanepentol	$C_5H_{10}O_5$	150	No significant report.
34.55	11.60	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	Antimicrobial, antioxidant.[10,35]
34.90	45.03	Dihydrochrysin	$C_{15}H_{12}O_4$	256	Antimicrobial, anti-inflammatory, anticancer, and neuroprotective activity.[26]
37.21	6.21	9-Hexadecenoic acid.	$C_{16}H_{30}O_2$	254	Anti-inflammatory protective effects on hepatic steatosis and insulin signaling in murine.[36]
38.39	2.10	Octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$	280.4	Anti-inflammatory, antibacterial, antiarthritic, hepatoprotective, anti-histaminic, anticoronary activity.[10]

DISCUSSION

The use of natural remedies in treating cancer has received much interest in the latest days, thanks to their diverse phytochemical compounds and numerous bioactivities.[19] Preliminary qualitative phytochemical analysis revealed the occurrence of a great variety of phytochemicals in the methanolic extracts of the leaf (Table 1). Other solvents were efficient in extracting different phytochemicals. This was determined by the comparative polarity and affinities of different compounds.[20] Plants' secondary metabolites possess distinctive biological activities. Tannins are utilized as an astringent as well as for the treatment of dysentery and diarrhea.[21] Alkaloids are a pharmacologically active category of natural chemicals and play important role in drug discovery. They were possesses various pharmacological activities which include anti-oxidant,

Table 3: *In-vitro* cytotoxic screening by *Macrosolen parasiticus* (L.) Danser leaf methanolic extract against PC- 3 cells by MTT assay.

Sl. No	Concentration µg/mL	Cell viability %	IC_{50} value	Standard (curcumin 10µM) cell viability %
1.	Untreated	100	448.7µg/mL.	50.26
2.	31.25	97.58		
3.	62.5	83.19		
4.	125	73.92		
5.	250	58.66		
6.	500	48.96		

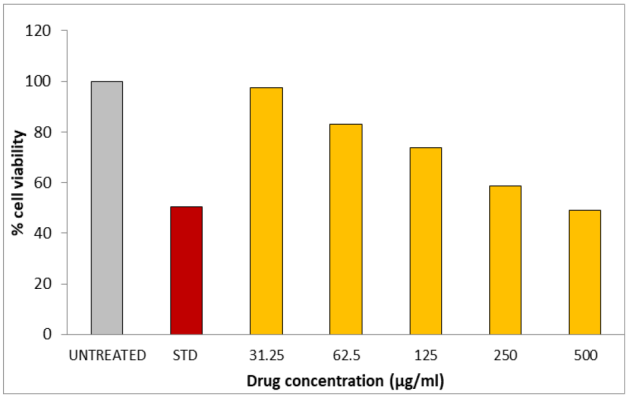

Figure 2: Cytotoxicity of *M. parasiticus* methanolic extract and standard drug on PC–3 cellline.

antihyperlipidemic, and antiobesity.[22] Just *et al.*, have reported saponins help in the anti-inflammatory activities of cells.[23] Steroids are significant because they interact with a variety of hormones in the body, and their antiviral properties have been proven.[8] Flavonols have antibacterial, antioxidant, analgesic, antiallergic, and anti-inflammatory effects.[24] These compounds have also been proven to be poisonous to foreign organisms and have been utilized to eliminate human cancer cells.[25]

GCMS analysis of the leaf methanol extract of *M. parasiticus* shows the presence of bioactive molecules. Among the identified phytocompounds 11 compounds were known for theirtherapeutic uses and the remaining 5 compounds were unknown for their therapeutic uses. Sodde *et al.*, found that *M. parasiticus* has substantial anticancer activity against Ehrlich's Ascites Carcinoma and MCF-7 breast cancer cells.[12-13] Puneetha *et al.*, earlier reported that it exhibits antioxidant properties.[11] Major recognized phytoconstituents with anti-cancer and antioxidant effects were identified in this investigation. Quattrocchi reported that *M. parasiticus* is used in ethnoveterinary remedies to eliminate ticks.[9] The compound n- Hexadecanoic acid has a potent pesticide and mosquito larvicidal property.

To evaluate toxicity, the MTT test is used to screen crude extracts and isolated chemicals. It could also indicate the cytotoxic properties of herbal samples.[39] Many plants in the Loranthaceae family have been shown to have powerful anti-cancer properties *in vitro* and also in animal models.[40-43]

Macrosolen parasiticus extracts showed antioxidant effects and anticancer activities on Ehrlich's ascites carcinoma and MCF-7 cell lines.[14-16] Plants having antioxidant activity inhibit the growth of a variety of human cancers, indicating anticancer potentials. Dietary polyphenols were shown to have anticarcinogenic properties in animals.[44] *in vitro* anticancer activity assay using Prostate cancer cell lines (PC-3) was used to analyze potentially harmful chemicals that affect cells' basic functioning and shape. Methanolic leaf extract of *M. parasiticus* inhibited the development of PC-3 cancer cell lines *in vitro*, whereas there were no effects on the growths of mice embryo fibroblast cell lines (MEF-L929). These selective effects depend on the concentrations and times of incubation. Concerning concentrations (31.25 µg/mL., 62.5 µg/mL., 125 µg/mL., 250 µg/mL., 500 µg/mL.) of each extracts were determined in duplicate by serial dilution. Among all five concentrations, 500 µg/mL of methanolic extract was the most potent in causing percent inhibition of growth. The results showed that methanolic extract moderately inhibited the PC-3 cancer cell lines at higher concentrations with IC_{50} values of 448.7µg/mL. As a result, methanolic leaf extracts may suppress cell proliferation due to various bioactive components, phenols, and other anti-oxidant agents found in *M. parasiticus*.[45]

CONCLUSION

It was concluded that *Macrosolen parasiticus* (L.) Danser possesses a wide range of phytochemical constituents, 16 phytocompounds were identified from the leaf methanolic leaf extract by GC-MS analysis. Among the identified compounds, the majority of compounds have a role in antioxidant, antimicrobial, anticancer, and anti-inflammatory effects. The methanolic leaf extracts show moderate cytotoxicity against the prostate cancer cell line. However, the isolation of individual phytochemical compounds and subjecting them to their anticancer potential improve their efficacy as a medicinal agent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GCMS: Gas Chromatography-Mass Spectroscopy Analysis; **MTT:** 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **MEF-L929:** Mice Embryo Fibroblast cell line; **NCCS:** National Centre for Cell Science; **PC-3:** Prostate Cancer cell line; **DMEM:** Dulbecco Modified Eagle Medium; **NIST:** National Institute Standard And Technology; **ELISA:** Enzyme-Linked Immunosorbent Assay; **IC_{50}:** inhibitory concentration at 50% growth; **NCCS:** National Centre for Cell Science; µg: microgram; **mL:** mili liter; **hr:** hour; **min:** minute; **nm:** nanometer.

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