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Phytochemical Screening and Mass Spectrometric Analysis to Unveil the Pharmacological Properties of Weed *Euryops pectinatus*

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Abstract

Euryops pectinatus 'Viridae' weed belonging to the family Asteraceae is commonly known as Golden Euryops or African daisy. Present investigation includes phytochemical analysis of methanol extract of *Euryops pectinatus* weed for the presence of various secondary metabolites. Presence of various secondary metabolites was observed including phenols, flavonoids, alkaloids, steroids, saponins, glycosides, etc. These secondary metabolites are well known for their biological activities like antioxidation, anti-cancerous, anti-microbial, cardiovascular, and anti-aging. After determination of rich secondary metabolites in the methanol extract, mass spectrometric analysis was carried out for the identification of compounds present in *Euryops pectinatus*. Eight compounds were identified in the methanol extract reported for nematicide, antioxidant, analgesic, antitumor, anticancerous and several other important activities. Thus, phytochemical and mass spectrometric analysis would open new doors to natural product research that would be cost effective and safe for the mankind.

Keywords: *Euryops Pectinatus*; Phytoconstituents; Bioactives; Mass-Spectroscopy; Anticancer

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Introduction

Weeds compete with crops for sunlight, water, nutrients, and space. In addition, they harbor insects and pathogens, which attack crop plants. Furthermore, they destroy native habitats, threatening native plants and animals [1]. *Euryops pectinatus* 'Viridae' belonging to the family Asteraceae is a weed and grows easily in full sun on well drained soil. Due to its spreading nature, it affects the growth of other agricultural crops by utilizing available

nutrition and space. However, special attention is given nowadays to unwanted weeds as they possess some special properties that make them grow easily under the abiotic stress conditions. Plants are a rich source of phytoconstituents that provide unlimited opportunities for the development of new drug leads. Therapeutic drugs identified from natural products are now acknowledged throughout the world.

Weeds are a serious problem in agriculture and tremendously reduce the productivity of

agricultural land by competing with crop plants for space and nutrition. Raipur District is highly occupied with weeds belonging to Asteraceae family, of which several are reported to have medicinal value. Natural products from plants provide unlimited opportunities for new drug leads thus the demand for therapeutic drugs from natural products has grown throughout the world. To overcome the problem of growing pest in agricultural fields and waste lands, and to utilize the locally available flora of the state, it is proposed to identify the bioactive metabolites of these unwanted crop plants and opening new doors for their pharmacological applications. In this proposed work new method is designed using the existing information concerning the use of Asteraceae weeds of Raipur region for identification of their bioactive metabolites that will be cost effective and safe for pharmacological applications.

Plant extracts are rich source of alkaloids, steroids, terpenes, antioxidant phenolics, flavonoids and other biologically active compounds. In modern medicine these compounds have been investigated for their medicinal applications [2]. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds [3]. Natural bioactive compounds like phenols and flavonoids are the important secondary metabolites in plants and possess biological properties like antioxidant, anti-aging, anti-carcinogen, protection from cardiovascular, immune/autoimmune diseases and brain dysfunctions viz. Parkinson's, Alzheimer's, Huntington's diseases, etc [4,5]. These active metabolites especially from herbs are the interest subject of research, and their phytochemical or biological investigations open new doors to the natural product research. The aim of present is to analyze the phytoconstituents and bioactive metabolites of Asteraceae weeds collected from the Raipur region with the objective of characterization of secondary metabolites using mass spectrometry.

Euryops pectinatus is endemic to rocky, sandstone slopes in the Western Cape of South Africa [6]. Chhattisgarh is commonly known as Bowl of rice, and in a study of the weed flora of dry land crops in the Chunkatta and Bhilai areas, Asteraceae was found to be second largest in species distribution, most of which may possess useful properties [7]. Another study have shown that highest number of weeds present in Durg district of Chhattisgarh state belongs to Acanthaceae, Asteraceae, Malvaceae and so on [8].

Materials and Methods

Collection and preparation of plant material

Plant material was collected from Raipur and identified as *Euryops pectinatus* 'Viridae' commonly known as Golden euryops. The samples were washed thoroughly in running tap water to remove soil particles and other adhered debris and finally washed with sterile distilled water. The whole plant was air dried for 24 hours and further used for extraction.

Plant sample extraction

Fresh leaves of *Euryops pectinatus* 'Viridae' (10g) were shade dried for 3-4 days and crushed using mortar-pestle. Extraction was done with methanol thrice for three consecutive days at 28°C (100 ml x 3). The extract was filtered twice with Whatman filter No. 1. Extract was combined and concentrated at 40°C in water bath. The crude methanol extract of *E. pectinatus* was stored at 8°C for further analysis.

Qualitative phytochemical analysis

Euryops pectinatus 'Viridae' methanol extract were tested for the presence of different classes of compounds [9-11]. Qualitative chemical test were used regarding the nature of phytoconstituents present in the methanolic extract (Table 1).

Table 1: Phytochemical analysis of explants of Golden Euryops.

Compounds	Test
Alkaloids	Dragendroff's Test
Glycosides	Salkowski's Test
Polyphenols and Tannins	Ferric Chloride Test
Flavonoids	Lead acetate Test
Proteins	Ninhydrin Test
Carbohydrates	Molisch's Test
Saponins	Foam Test
Steroids	Steroids Test
Quinones	Quinones Test
Coumerin	Coumerin Test
Terpenoids	Salkowski Test
Phlobatannins	Phlobatannins Test
Phytosterol	Libermann's Test

Gas Chromatography- Mass Spectrum analysis (GC-MS)

Characterization of crude methanol extract of *Euryops pectinatus* 'Viridae' was done by Gas Chromatography- Mass Spectrometry. GC-MS was recorded in a GCMS-2010 Shimadzu instrument operating in EI mode at 70eV. A Restek-5MS column (30m x 0.25mm x 0.25µm) was used. The oven temperature program was 100^o to 250^oC at 5^oC min⁻¹ and held for 5 min at 250^oC and from 250^oC to 280^oC at 10^oC min⁻¹ and held for 10 min at 280^oC. The injector temperature was 250^oC with normal injection mode. The flow rate of carrier gas helium was 1.21ml min⁻¹. The identification of methanol extract was confirmed by comparing the mass spectral data with those of authentic compounds and with data obtained from the literature.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

The preliminary phytochemical screening of the methanolic extract of leaves of *Euryops pectinatus* 'Viridae' showed the presence of various secondary metabolites of which alkaloids, flavonoids, terpenoids, saponin glycosides, tannin and phenolics compounds were the most prominent (Table 2).

Table 2: Preliminary phytochemical analysis of the methanol extract of Golden Euryops.

Compounds	Golden Euryops (MeOH)
Alkaloids	+++
Glycosides	++
Polyphenols and Tannins	+++
Flavonoids	+++
Proteins	+
Carbohydrates	++
Saponins	+
Steroids	++
Quinones	-
Coumerin	+
Terpenoids	++
Phlobatannins	-
Phytosterol	+

+++ Maximum, ++ Moderate, + Least presence, - Absence of the compound

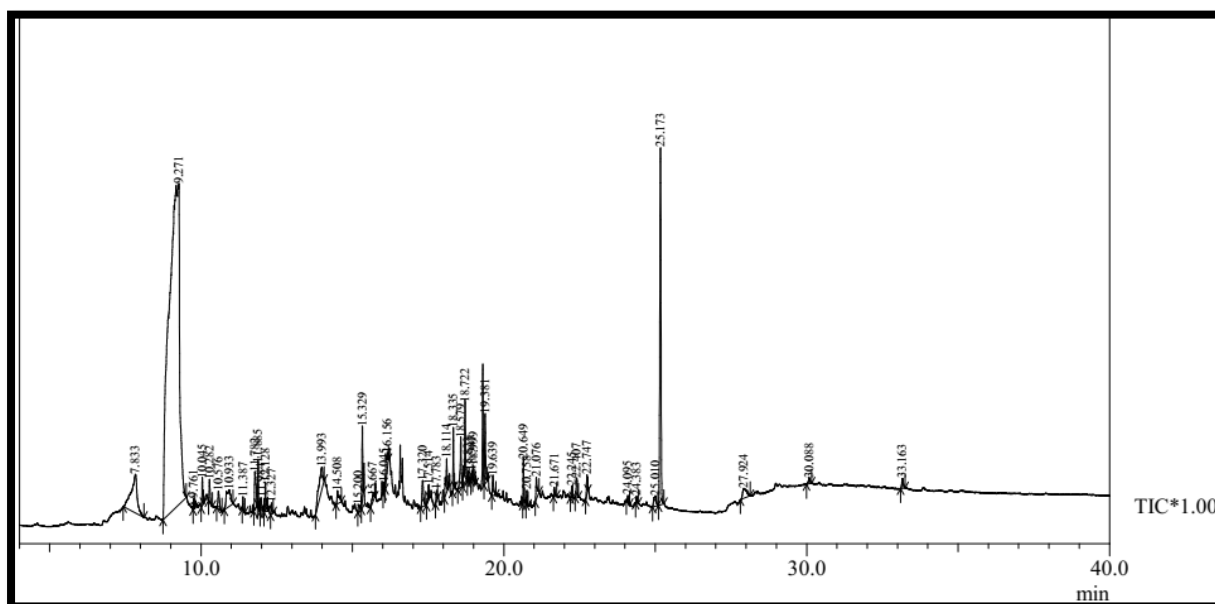


Figure 1: Mass spectrometric analysis of methanol extract of *Euryops pectinatus* 'Viridae'.

Table 3: Bioactive compounds identified in the methanol extract of leaves of Golden *Euryops*.

S. No.	R. Time	Name	Mol. Formula	Mol. Weight	Area%
1	10.576	1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-l-rhamnitol	C ₁₄ H ₂₂ O ₈	318	0.53
2	12.870	7,7A-dimethyl-3A,4,5,7A-tetrahydro-3H-benzofuran-2-one	C ₁₀ H ₁₄ O ₂	166	1.91
3	15.667	Propionamide, N-(3-fluoro-2-methylphenyl)-3-(4-fluorophenyl)	C ₁₆ H ₁₅ F ₂ NO	275	0.30
4	16.217	Tau-Muurolol	C ₁₅ H ₂₆ O	222	0.76
5	16.580	Patchoulane	C ₁₅ H ₂₆	206	1.06
6	18.722	Aniline, N-(2-(diethylamino)ethyl)-2,4-dinitro-	C ₁₂ H ₁₈ N ₄ O ₄	282	1.64
7	25.010	Morpholine, 4,4'-(phenylmethylene)bis	C ₁₅ H ₂₂ N ₂ O ₂	262	0.49
8	25.173	1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390	8.00

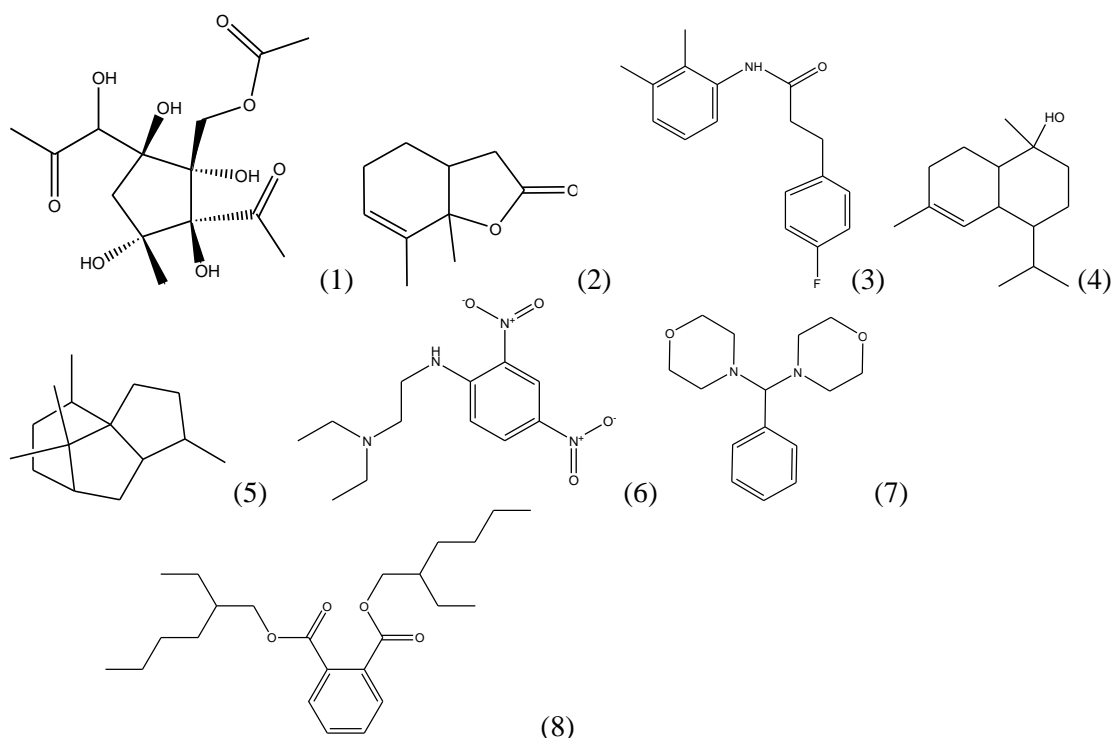


Figure 2: Compounds identified in the methanol extract of leaves of *Euryops pectinatus* ‘Viridae’ by GC-MS.

Table 4: Biological activity of the compounds identified in the methanol extract of leaves of Golden euryops.	
Name	Activity*
1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-1-rhamnitol	Anticancer (oral), Antitumor (Ovary), Inhibit Production of Tumor Necrosis Factor
7,7A-dimethyl-3A,4,5,7A-tetrahydro-3H-benzofuran-2-one	Anti-HIV Integrase, Antidote (Hydrazine, Heavy Metals, Hypoglycin A)
Propionamide, N-(3-fluoro-2-methylphenyl)-3-(4-fluorophenyl)	Antitumour, Anaphylactic, Increase NK cell activity, Narcotic, Nematicide, Neuroinhibitor, Neuroprotective, Neurostimulant, Neurosedative
Tau-Muurolol	Anticancer (skin, stomach), Antidiabetic, Antidote (snake venom, strychnine, Salicylate), Antioxidant synergist, Antitumor (Skin, stomach)
Patchoulane	Anti-HIV Integrase, Antidote (Hydrazine), Anaphylactic, Analgesic, Antiaging, Anti-IgE, Antiacne, Anticancer
Aniline, N-(2-(diethylamino)ethyl)-2,4-dinitro-	Nematicide, Anaphylactic, Antitumor, Increase NK cell activity, Narcotic
Morpholine, 4,4'-(phenylmethylene)bis	Increase SOD Activity, Disinfectant, Antidote (Diazepam, Digoxin)
1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Arachidonic acid- Inhibitor
*Source: Dr. Duke’s Phytochemical and Ethnobotanical Databases (online database).	

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extract [12]. The spectrum for the phytochemicals of the methanolic leaf extract of Golden euryops was determined using GC-MS (Figure 1).

Eight compounds are reported to have biological activity (Figure 2, Table 4). The activity prediction is based on Dr. Duke's Phytochemical and Ethnobotanical databases and available literature. Compound 1,4-Diacetyl-3-acetoxymethyl-2,5-methylene-1-rhamnitol is also reported in the methanol extract of *Aspergillus terreus* that showed antibacterial and anti-fungal activity [13]. Similarly another compound a Patchoulane-type Sesquiterpene, 6-Acetoxy Cyperene isolated from *Cyperus rotundus* rhizomes is reported to induce caspase-dependent apoptosis in human ovarian cancer cells [14].

Conclusion

In the present study, methanolic extract of *Euryops pectinatus* 'Viridae' commonly known as Golden euryops, a weed was analysed using mass spectrometry. Through these results, we can conclude that *E. pectinatus* can be exploited to isolate and characterize important bioactive compounds for pharmaceutical and industrial applications. Several phytochemicals from plants have recently attracted a great deal of attention, mainly due to their role in anticancerous, antimicrobial, antioxidant properties etc. At present there are so many economically important plant species that are being utilized for their important secondary metabolites. Plants contain secondary metabolites meant for defense against various pathogens. Identification of local weeds belonging to Asteraceae family containing bioactive potential against microorganisms, cancer, etc would open new doors in the field of

pharmacology, as use of plants are safe and cost-effective.

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