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Isolation And Characterization Of Sulfur Containing Compounds In Cleome Genus And Activity Analysis; A Comparative Analysis In *Cleome viscose* and *Cleome gynandra.*

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ABSTRACT

A variety of phytochemicals known as primary and secondary metabolites are secreted by plants. Primary metabolites are frequently ingested and are necessary for plant physiology. On the other hand, secondary metabolites are bioactive substances and do not necessarily need to be used by plants. The therapeutic potential of secondary metabolites and nutraceuticals has both been examined recently. Two plant species from the Cleome genus, Cleome gynandra and Cleome viscose, were found and employed in the current work to extract secondary metabolites. The secondary metabolites were extracted using three solvents: methanol, water, and chloroform. Antioxidant activity of isolated compounds was examined and determined using the DPPH assay (DPPH radical scavenging assay). Antibacterial activity of methanolic extract was determined via plate diffusion assay.Higher concentrations of the secondary metabolites were detected in the methanolic extract of Cleome gynandra and Cleome viscose leaves. The methanolic extract's anti-inflammatory and anti-malarial properties were investigated. Both Cleome gynandra and Cleome viscose have shown in the current investigation that methanolic and chloroform extracts have strong antibacterial and antioxidant activity.

Keywords; Sulfur, secondary metabolites, *Cleome viscose and* Cleome *gynandra,* extraction, antioxidant and antimicrobial activity.

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INTRODUCTION

Plants produce secondary metabolites to increase their competitiveness within their respective ecosystems. These tiny chemicals have a variety of effects on both the plant and other living things. They either maintain perennial growth or indicate deciduous behavior [1]. They also cause flowering, fruit set, and abscission. They serve as attractants or, alternatively, repellents and act as antimicrobials. Secondary plant metabolites are the basis for the therapeutic effects of many modern medications and medicinal plants. The plant kingdom produces hundreds of thousands of low molecular weight organic compounds [2]. Based on the assumed functions of these compounds, the research community has classified them into three overarching groups: primary metabolites, which are directly required for plant growth; secondary (or specialized) metabolites, which mediate plant–environment interactions; and hormones, which regulate organism processes and metabolism [3]. For decades, this functional trichotomy of plant metabolism has shaped theory and experimentation in plant biology. However, exact biochemical boundaries between these different metabolite classes were never fully established. A new wave of genetic and chemical studies now further blurs these boundaries by demonstrating that secondary metabolites are multifunctional; they can function as potent regulators of plant growth and defense as well as primary metabolites [4]. Several adaptive scenarios may have favored this functional diversity for secondary metabolites, including signaling robustness and cost-effective storage and recycling [5].

Secondary metabolite multifunctionality can provide new explanations for ontogenetic patterns of defense production and can refine our understanding of plant–herbivore interactions, in particular by accounting for the discovery that adapted herbivores misuse plant secondary metabolites for multiple purposes, some of which mirror their functions in plants [6]. To safely improve human and animal health, medicinal plants can be used as a natural source of therapeutic medications, nutraceuticals/food supplements, and feed additives [7]. In an effort to combat germs that are multidrug resistant, there has been a surge in interest in looking into plants as a potential new source of various pharmaceuticals, particularly antimicrobials [8]. The Cleome genus is one of the largest genera among medicinal plants and is a member of the Cleomaceae family. About 180–200 species fall under this genus, which is geographically dispersed over Egypt, Libya, Palestine, Syria, and other arid and semi-arid regions [9]. They are furthermore perennial, low, fragrant cushion-shaped shrubs with a length of 25–60 cm that have intricately branching stems and broad, oblong, three-nerved leaves with enlarged glandular hairs. This genus of shrubs has medicinal and ecological significance [10-11]. For treating stomachaches, skin allergies, and open wounds as well as having anticancer and hepatoprotective qualities, shrubs of the cleome species are well-known in folk medicine. The aqueous extract of Cleome has been found to possess a very high percentage of flavonols that showed 63.3% action, similar to that of the synthetic medicine metformin.

In addition, the Cleome genus of shrubs has demonstrated excellent antidiabetic characteristics. Shrubs from the cleome genus exhibit antibacterial, antiparasitic, and antioxidant properties [12]. These biological effects are connected to the wide variety of secondary metabolites that are present naturally in shrubs belonging to the Cleome genus. From plants in the Cleome genus, several terpenes, flavonoids, glucosinolates, anthocyanin alkaloids, and polyphenols have been identified [13]. Given these biological functions of the shrubs of the Cleome genus, more research is needed to investigate the active secondary metabolites of these shrubs and their potential as novel natural feed and dietary supplements for enhancing both animal and human health. Hence, in the present study, forest area of Southern India was exploring for plants species for the isolation of secondary metabolites. In the present study, secondary metabolites with sulfur containing compounds were targeted for analysis. The study also emphasizes antioxidant and antimicrobial activity of Cleome genus from forest areas of Southern India.

MATERIALS AND METHODS

Collection of plants and secondary metabolites extraction

The southern Indian forest in Tamil Nadu and Andhra Pradesh were selected for the collection of plant species. From the forests area of East Godavari, Andhra Pradesh, Munnar, and Kodaikanal, Tamil Nadu, the leaves and roots of *Cleome gynandra* and *Cleome viscose* were harvested. In the present study, southern India was selected for the collection of plants species as extended

biodiversity. Two prominent states of India Tamil Nadu and Andhra Pradesh were selected for the collection of the plant species. The forest area of Munnar and Kodaikanal, Tamil Nadu, and Godavari (East) of Andhra Pradesh were spotted for collection of plant species. The plants were identified based on botanical phenotypes and plant parts (leaves and roots) were collected. *Cleome gynandra* and *Cleome viscose* species differ in flower characteristics and using those properties both the plant were collected separately. In the present study, 2000 gm of leaves and roots from *Cleome gynandra and Cleome viscose* were collected from multiple sites of forest area of selected sites. The plant parts were wrapped into collection bag to avoid loss of humidity and volatile phytochemicals. The leaves and roots collected for *Cleome gynandra and Cleome viscose* species were grouped and labeled. Here, drying of leaves and roots was carried out in two separate steps; one air dry and second under vacuum. The dried leaves and roots of *Cleome gynandra and Cleome viscose* species were subjected to grinding using pestle mortar. The weight was measured for leaves and roots of each group before drying and after drying as well. The leaves and roots after grinding moisture content was calculated and subjected to extraction. For the extraction of secondary metabolites and S containing secondary metabolites precisely 50gm of fine grinded power from each group was loaded to Soxhlet apparatus separately in the presence; Methanol, Chloroform and water.

Culture and Microorganisms

The bacterial strains employed in the current study were procured from Microbial Type Culture Collection and Gene Bank (MTCC) a national facility of microbial species at Institute of Microbial Technology (IMTECH), Chandigarh India. The bacterial species for antimicrobial study includes *Bacillus subtilis, Pseudomonas species, Staphylococcus aureus, Klebsiella pneumonia and Proteus mirabilis.* These bacterial species were supplied as lyophilized form and re-suspended into sterile water as per instructions made by supplier. The bacteria species were grown in nutrient rich media for 24-48 hour at 37^0C. The secondary culture was made as per media recommended by supplier and grown overnight at 37^0C. The overnight grown culture was used to grow bacterial species on plates and single colony was picked up. The glycerol stock was prepared for long term storage and culture from single colony was used for antimicrobial test. Nutrient agar and broth that contain peptic digest of animal tissue (5g/L), yeast extract (1.50g/L), Beef extract (1.5g/L) was used for the growth of bacterial cultures. Antibiotic assay media containing peptic digest of animal tissue (6g/L), casein enzyme hydrolyte (4g/L), Yeast extract (1.50g/L), Dextrose (1.00g/L). Agar (15.00g/L) was used for anti-bacterial activity. Additionally, media used for bacterial growth and maintenance is Luria broth and agar. Both nutrient broth and agar medium were used for subculture and maintenance of bacterial strain. The medium was prepared with the following composition for one-liter volume as Beef extract 1.0g/l, Yeast extract 2g/l, Peptone 5g/l, Sodium Chloride 5g/l and agar 15g/l. The medium was prepared in distilled water, and pH was adjusted 7 by using concentrated HCl. The media was sterilized by autoclaving at 121psi for 20min.

Evaluation of *In vitro* Antioxidant activity

Cleome gynandra and Cleome viscose root and leaves extracts were tested for antioxidant content using the DPPH radical scavenging assay. An antioxidant assay based on electron transfer, the DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical approach generates a violet solution in ethanol. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, is reduced, producing a colorless ethanol solution. The DPPH assay offers a simple and quick method for spectrophotometer-based antioxidant evaluation. Dimethyl sulfoxide (DMSO) was used to dissolve the *Cleome gynandra and Cleome viscose* root and leaves extracts to provide a stock solution (10 mg/mL) for antioxidant tests. The DPPH radical solutions were made with 95% ethanol and a concentration of 120 µM. [14]. *Cleome gynandra and Cleome viscose* root and leaves aqueous extracts were created by dilution twice in 96-well microtitre plates for the antioxidant assay. In 96-well microtitre plates, an aliquot of the extract (10µl) was combined with 195 µl of methanolic DPPH. A spectrophotometer was used to measure the reaction mixtures' absorbance at 517 nm after they had been incubated at room temperature for 30 minutes in the dark. The effective concentration at which DPPH radicals were scavenged by 50% is known as the IC50 value (g/mL), and it was calculated by interpolating the results of the linear regression analysis [15]. The free radical scavenging activity was calculated as follows:

% Free Radical Scavenging activity = [(Blank – Sample / Blank] ×100%

Where: Blank was the absorbance of without samples, and Sample was the absorbance of the test sample. The values are expressed as the means of triplicate analyses. The antioxidant activity is a function of percentage inhibition calculated for both extracts collected from both species of the *Cleome gynandra and Cleome viscose* root and leaves.

DPPH radical scavenging assay

Blois originally described the DPPH radical scavenging assay, which was used to measure the extracts' antioxidant activity (1958). Because of the paramagnetic properties provided by an odd electron, DPPH (1, 1-diphenyl-2-picrylhydrazyl) is regarded as a stable radical (delocalization of the spare electron over the molecule as a whole). The solution exhibits a prominent absorption band at 520 nm and is deep violet in color (in 100% ethanol). The pale violet DPPH radical can take an electron or hydrogen radical to form a stable diamagnetic molecule. When an anti-oxidant testing material is combined with DPPH solution and turns pale violet, this indicates that the anti-oxidant action of the substance is due to free radical scavenging activity. The test method outlined below was adapted from that of previous studies [16-17]. Dissolved methanol, $CHCl_3$ and ethyl acetate extracts in absolute ethanol and water extract in distilled water. Diluted each sample for at least 5 concentrations (two-fold dilutions).Prepared 6x10-5µM of DPPH in absolute ethanol. Transferred 500µl of each sample solution into an eppendroff tube. Each concentration was tested in triplicate. Transferred 500µl of DPPH solution to mix with sample solution. Shake and stand at the room temperature for 30 minutes. Measured absorbance at 520 nm, using a mixture of 500 µl sample solution and 500 µl absolute ethanol as blank. Prepared standard solution and control in each experiment as follows:-Control ethanol: mixture of absolute ethanol (500 µl) and 6x10-5µM DPPH in absolute ethanol (500 µl); blank: absolute ethanol. Control water: mixture of distilled water (500 µl) and 6x10-5 M DPPH in absolute ethanol (500 µl); blank: mixture of distilled water (500 µl) and absolute ethanol (500 µl). Plotted dose-response curve between % inhibition and concentrations. Linear regression analysis is carried out for calculating the effective concentration of sample required to scavenge DPPH radical by 50 % (ED50 value). In each experiment Quercetin, a well known natural antioxidant is used as the positive control.

% inhibition=OD control-OD sample x 100/OD control

GS-MS based characterization of Sulfur containing compounds

The mixture of compounds in the mobile phase interacts with the stationary phase. Each compound in the mixture interacts at a different rate. Those that interact the fastest will exit (**elute** from) the column first. Those that interact slowest will exit the column last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated. Further refinements to this separation process can be made by changing the temperature of the stationary phase or the pressure of the mobile phase. Our GC has a long, thin fused silica column containing a thin interior coating of a solid stationary phase. This 0.25 mm diameter column is referred to as a capillary column. This particular column is used for semi-volatile, non-polar organic compounds. The compounds must me in an organic solvent. As the individual compounds elute from the GC column, they enter the **electron ionization** (mass spec) detector. We have profiled methanolic extract of *Cleome gynandra and Cleome viscose* root and leaves extracts using GCMS analysis. The dried powder of *Cleome gynandra and Cleome viscose* root and leaves aqueous extracted in different solvent with increasing polarity such as chloroform, ethyl acetate, and methanol. The GC instrument was allowed for a calibration and heating of column. The methanol was run through column until a baseline was recorded. The column remains heated and instrument was calibrated. We have used here JASCO gas chromatography instrument connected with a mass spectroscope. Both the instruments were calibrated as per instruction given by supplier. *Cleome gynandra and Cleome viscose* root and leaves aqueous extracts nearly 2µl was injected into GC sample inlet. The sample was allowed to mix with carrier gas and travelled through column. Here, we run multiple samples of *Cleome gynandra and Cleome viscose* root and leaves aqueous extracts from both wild and hybrid species. The volume of sample inlet was constant and equal in both cases.

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RESULTS AND DISCUSSION

The plants were collected from forest area of Munnar and kodaikanal, Tamil Nadu, India. Plants collected from forest area were grouped into two category and different parts including leaves, roots and stem were collected. We have collected 2.5 kg of plants parts from both the plant species i.e. *Cleome viscose and Cleome gynandra*. Collected plant species belongs to the Cleome genus with the several morphological features. Cleome species are annual or perennial herbs. Plants parts were subjected to drying; air dry and vacuum dry. Plant parts from both the species *Cleome viscose and Cleome gynandra were* grinded and subjected to the soxhet apparatus for extraction. Here we have used three major solvent for the extraction of the secondary metabolites including sulfur containing compounds. The plants part from both species *Cleome viscose and Cleome gynandra were* subjected to the extraction.

Extraction of phytochemicals

The extraction of secondary metabolites from *Cleome gynandra and Cleome viscose* were successfully carried out and characterized. The yield of extraction was calculated for *Cleome gynandra and Cleome viscose* among all solvents and both plant parts leaves and root. We report here maximum extract yield with methanol in both the species of *Cleome gynandra and Cleome viscose* i.e. 42.89 % and 46.11%, respectively. The yield was calculated as the formula given above. It has been reported that leaves of *Cleome gynandra and Cleome viscose* possess higher amount of active secondary metabolites than other solvents (chloroform and water). Further, extract from both the species of *Cleome gynandra and Cleome viscose* demonstrated the presence of active phytochemical constituents, as shown in the antibacterial and antioxidant analysis. Our findings show the presence of active metabolites in *Cleome gynandra and Cleome viscose* extracts and shown higher extraction yield from previous studies. The extraction of active secondary phytochemicals from *Cleome gynandra and Cleome viscose* including leaves and roots using different solvent determine activity profile.

Table 1: The chart represents the antibacterial activity of *Cleome viscose and* Cleome *gynandra* extract from the different solvent with control. The antibacterial activity was measured as a function of the zone of inhibition.

Bacterial Species	Zone of Inhibitions (mm)							
	MCG	MCV	WCG	WCC	CCG	CCV	Positive Control	Negative Control
Bacillus subtilis	23	22	19	19	13	15	26	11
Pseudomonas Species	21	23	21	22	14	15	25	13
Staphylococcus aureus	20	19	25	20	15	19	30	10
Klebsiella pneumoniae	19	20	23	19	11	15	27	13
Protieus mirabilis	18	20	20	20	16	19	27	12

IC 50 Values

The IC50 value represents a minimum concentration of an antimicrobial or antibiotic to inhibit bacterial growth. We reported here the extract of *Cleome viscose and* Cleome *gynandra* primarily methanolic extract posses an IC 50 21.20 µg/ml and 20.47 µg/ml respectively (Table 2). Other extracts were reported with non-significant IC 50 including water and Chloroform. These finding again confirms that the active phytochemicals present in *Cleome viscose and Cleome gynandra* leaves and roots have an affinity for methanol. These compounds do have an affinity for other solvent but non-significant.

Table 2: IC 50 values of standard and extract (Methanolic *Cleome viscose and Cleome gynandra*).

Sample	IC 50 Value
Standard	25.91 µg/mL
Sample (MCV)	21.20 µg/mL
Sample (MCG)	20.47

Antioxidant activity

Plants and plant derived products are rich in active phytochemicals. These molecules exert several therapeutic and protective properties to plants including antimicrobial and antioxidant. The antioxidant property of *Cleome viscose and* Cleome *gynandra* extract of leaves and root was determined using DPPH radical scavenging assay. The ascorbic acid was used to prepare a standard for DPPH radical scavenging assay, and based on the standard curve antioxidant activity of *Cleome viscose and* Cleome *gynandra* extract was calculated. As a result, shown in table 3, we report here maximum DPPH inhibition was reported with methanol extract of Cleome *gynandra leaves*. However, *Cleome viscose* with methanol also demonstrated a significant DPPH inhibition compare to another solvent. The higher content of flavanoids and another phytosterol, along with vitamin C, are responsible for the antioxidant activity *Cleome viscose and* Cleome *gynandra*. As a result, shown in table 4, it is evident that phytochemicals responsible for antioxidant properties have more affinity for methanol. As a result, shown in a recent finding **Rehman, 1990**, we report a similar finding [18]. Previously **Zarghami et al., 2021** demonstrated active phytochemicals present in *Cleome genus*, offering a vigorous antioxidant activity [19].

Table 3: The table represents the antioxidant potential of phytochemicals constituent present in *Cleome viscose and* Cleome *gynandra* using DPPH radical scavenging assay.

Conc. µg/ml	Methanolic Extract			
	Cleome *gynandra*		*Cleome viscose*	
	Absorbance	% DPPH inhibition	Absorbance	% DPPH inhibition
0	0.0711	0	0.0631	0
5	0.2154	6.35	0.1942	6.21
10	0.1358	11.52	0.1287	11.24
15	1.6584	16.36	1.4146	16.08
20	0.6584	21.35	0.5956	21.21
25	0.12485	26.35	0.1374	26.208
30	1.9254	32.32	1.8352	31.68
40	1.2154	42.65	1.1042	41.88
50	1.7125	51.32	1.6185	50.65

In our finding, we report here a large variety of *Cleome viscose and* Cleome *gynandra* phytochemicals might be responsible for intense antioxidant activity analyzed using DPPH free radical scavenging assay. **Hashem and Shehata, 2021** also investigated Antioxidant and Antimicrobial Activity of *Cleomedroserifolia* (Forssk.) and we found presence of phenolic compounds in Cleome genus is key secondary metabolite for the antioxidant activity [20].

Superoxide free radical scavenging activity

The results of superoxide radicals scavenging activity of chloroform, methanol and aqueous extracts of Cleome *viscose and* Cleome *gynandra leaves*. All extracts of the *Cleome viscose and* Cleome *gynandra* leaves extract at all tested concentrations showed free radical scavenging activity. Further the activity was found to be dose dependant. The IC_{50} value of the standard was found to be 0.86 µg/ml. The percentage inhibition of the standard was found to be 63.25%. The IC50 values of the extracts were found to be 12.41, 2.14 and 5.95 µg/ml .The percentage inhibition of methanol and aqueous extracts of *Cleome gynandra leaves* were found to be 89.54 and 57.63. The aqueous and methanol extracts of the leaves of Cleome *gynandra leaves* showed potent antioxidant activity. Chloroform extract also exhibited antioxidant activity but was less when compared to the methanolic extract (Table 4and figure 1). MCG has potent antioxidant activity among the three extracts as indicated by the IC_{50} value.

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Table 4: Superoxide free radical scavenging activity of *Cleome gynandra* leave extracts.

S. No	Test (extract)	Dose (µg/ml)	Percent inhibition	IC50 µg/ml
1	CCG	0.5	41.16	12.41
		1.0	52.65	
		2.5	76.32	
2	WCG	0.5	29.54	2.14
		1	39.54	
		2.5	57.63	
3	MCG	1	21.54	5.95
		2.5	49.25	
		5	89.54	
Standard Drug	Gallic acid	0.25	24.13	0.86
		0.5	44.79	
		1	63.25	

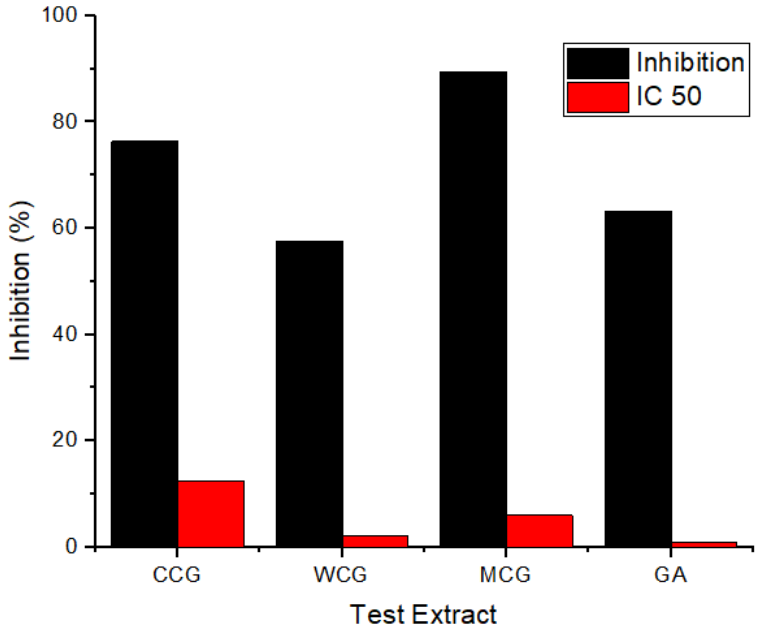

Figure 1: The figure demonstrates the free radical scavenging activity of *Cleome gynandra* leave extracts with inhibition of free radical.

Table 5: Superoxide free radical scavenging activity of *Cleome viscose* leaves extract.

S.No	Test (extract)	Dose (µg/ml)	Percent inhibition	IC50 µg/ml
1	CCV	0.5	33.74	11.16
		1.0	47.65	
		2.5	68.21	
2	WCV	0.5	27.58	1.98
		1	37.24	
		2.5	55.47	
3	MCV	1	39.21	4.47
		2.5	50.32	
		5	73.47	
Standard Drug	Gallic acid	0.25	22.32	0.74
		0.5	41.52	
		1	61.36	

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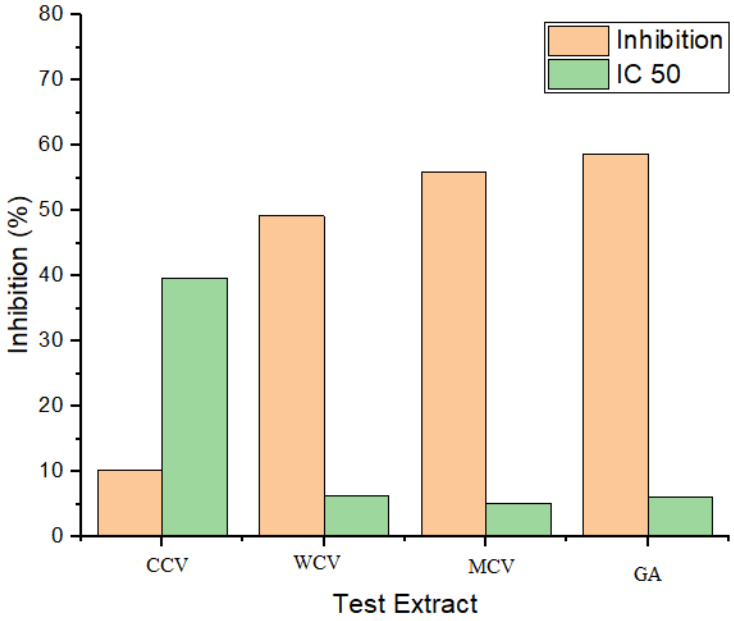

Figure 2: Superoxide free radical scavenging activity of *Cleome viscose* leaves extract.

Here the data showed in the table 8 and presented in the figure 4.8 the leaves of Cleome viscose. Further the activity was found to be dose dependant. The IC_{50} value of the standard was found to be 0.74 µg/ml. The percentage inhibition of the standard was found to be 61.36%. The IC50 values of the extracts were found to be 11.16, 1.98 and 4.47 µg/ml .The percentage inhibition of methanol and aqueous extracts of *Cleome viscosa leaves* were found to be 73.47 and 55.47. The aqueous and methanol extracts of the leaves of Cleome *viscosa* leaves showed potent antioxidant activity. Chloroform extract also exhibited antioxidant activity (superoxide free radical scavenging activity) but was less when compared to the methanolic extract (Table 5 and figure 2). MCG has potent antioxidant activity among the three extracts as indicated by the IC_{50} value.

Sulfur compounds containing secondary metabolites in *Cleome viscose* and *Cleome gynandra*

The GC-MS chromatogram peak report of methanolic extract of *Cleome viscose* and *Cleome gynandra* leaves are shown in table 6. As the result shown here based on retention time and abundance 30 different major phytochemicals were reported. The abundance as % of compound in sample was key parameter in listing major phytochemical in both *Cleome viscose* and *Cleome gynandra* leaves methanolic extract. In case of *Cleome viscose* methanolic extract aromatic compounds with sulfur were present in large amount than *Cleome gynandra*. However, various organic acids such as Acetic acid and corresponding alcohols were also reported entirely different from previous studies [21-22]. The aliphatic short chain compounds such as butane and derivatives were also reported in methanolic *Cleome viscose* and *Cleome gynandra*. These finding clearly suggests that being a polar solvent methanol served as ideal for extraction of active phytochemicals from *Cleome viscose* and *Cleome gynandra*. The diverse phytochemicals in *Cleome viscose* and *Cleome gynandra* clearly demonstrates wide range of therapeutic benefits [23-24]**.** The major phytochemicals/secondary metabolites that contain sulfur identified in *Cleome viscose* and *Cleome gynandra* leaves methanolic extract are summarized in the table 6. The plant species in *Cleome viscose* and *Cleome gynandra reports* 2-Methyl-1,3-thiazole, Ethyl 3-methyl sulfanyl propanoate, Glucocleomin, 5-Methylthiophene-2-carbaldehyde (5-methyl-2-formyl thiophene) and 5-ethyl-5-methyl-oxazolidine-2-thione [25]. The abudence of these listed sulfur compounds differ in *Cleome viscose* and *Cleome gynandra* methanolic extract.

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Table 6: Table summarizes the key sulfur containing compounds as secondary metabolites from the *Cleome viscose and Cleome gynandra.* The table also provides a comparative analysis of key secondary metabolites with sulfur for molecular formula, molecular weight, retention index, and abundance as percentage.

Name of Compound	*Cleome viscose*				*Cleome gynandra*			
	MF	MW	RI	%	MF	MW	RT	%
2-Methyl-1,3-thiazole	$C_5H_5NO_2S$	143.17	842	0.12	$C_5H_5NO_2S$	143.17	846	0.18
Ethyl 3-methyl sulfanyl propanoate	$C_6H_{12}O_2S$	148.23	863	0.32	$C_6H_{12}O_2S$	148.23	850	0.60
Glucocleomin	$C_{12}H_{23}NO_{10}S_2$	405.4	914	0.98	$C_{12}H_{23}NO_{10}S_2$	405.4	916	1.202
Glucocapparin	$C_8H_{15}NO_9S_2$	333.3	945	0.85	$C_8H_{15}NO_9S_2$	333.3	968	0.93
5-Methylthiophene-2-carbaldehyde (5-methyl-2-formyl thiophene)	C6H6OS	126.17	1124	0.63	C6H6OS	126.17	1170	0.72
5-ethyl-5-methyl-oxazolidine-2-thione	$C_6H_{11}NOS$	145.23	1207	0.52	$C_6H_{11}NOS$	145.23	1280	0.58

Presence of listed the compounds contains sulfur was reported significant higher in the *Cleome gynandra* over the *Cleome viscose* leave methanolic extract [26]. *Cleome viscose* and *Cleome gynandra also rich in the g*lucosinolates. GIs are a class of natural compounds which include sulfur and nitrogen and are derived from glucose and an amino acid. They are water-soluble anions and can be leached into the water during cooking [27]. Every Gls. contains a central carbonatom, which is bound to the thioglucose group, a nitrogen atom attached to a sulfate group and a side group. Different Gls. have different side groups which upon hydrolysis gave different products and the variation in the biological activities of these compounds due to its variation in their side chains. Cleome species are often used as spices as raw or powdered [28]. Cleome species are used for curing inflammation, wounds, pyrexia, pain; depression and diarrhea in extract form aerial parts of Cleome genus plants (especially *Cleome gynandra*) are also effective in the treatment of bacterial and fungal infections [29]**.** Cleome viscosa extracts showed Larvicidal and ovicidal activity in Aedes aegypti.

CONCLUSION

In the current study, the Cleome genus, which is native to southern India's Tamil Nadu and Andhra Pradesh, was looked at as a possible source of plant species for the isolation and characterization of secondary metabolites. Based on botanical criteria, *Cleome gynandra and Cleome viscose* were collected and identified. Secondary metabolites were extracted from the leaves and roots of Cleome gynandra and Cleome viscose using methanolic, aqueous, and chloroform as solvents. In the current investigation, phenolic secondary metabolites with antioxidant and antibacterial properties were found in significant amounts in the methanolic extract of plant species, *Cleome gynandra and Cleome viscose*. In the present study, six sulfur containing compounds were isolated and characterized. For the molecular research, the secondary metabolites were further described after being extracted and purified. The assessment of the clinical potential of secondary metabolites from the *Cleome genus* requires thorough in vivo and animal investigations.

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