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In-vitro assessment of antioxidant and cytotoxic activity of ethyl acetate extract of *Commiphora mukul*

Siva, S, L. Kalaiselvi, N Pazhanivel and R Gokul

Department of Veterinary Pathology

Madras Veterinary College, TANUVAS, Chennai-600 007

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Abstract

Commiphora mukul (Guggul), native to India, Pakistan, and Bangladesh, is widely used in traditional medicine for cancer, atherosclerosis, rheumatism, and obesity. This study evaluated the phytochemical composition, antioxidant activity and cytotoxic potential of its ethyl acetate extract. Phytochemical analysis confirmed alkaloids, flavonoids, phenols, tannins, steroids, and terpenoids while saponins, glycosides, cyanin, and proteins were absent. The extract showed high flavonoid content (84.54 mg CE/g) and lower phenolic content (9.76 mg GAE/g). DPPH and reducing power assays confirmed strong antioxidant activity. Cytotoxicity evaluated by MTT assay showed 89.24% inhibition at 1 mg/ml, with an IC50 of 46.67 µg/ml, indicating significant therapeutic potential.

Keywords: Antioxidant, *Commiphora mukul*, MTT assay , Phytochemicals

Introduction

Commiphora mukul (Guggul) is an ancient Ayurvedic medicinal plant from India, valued for its aromatic resin and therapeutic properties. A member of the *Burseraceae* family, it thrives in arid and semi-arid regions including Rajasthan, Gujarat, Madhya Pradesh, Karnataka as well as parts of Pakistan, demonstrating adaptability to varied soils and climatic conditions. It was categorized as Vulnerable in the 1997 IUCN Red List and later as reclassified as data Deficient in 2004. Owing to high international demand and conservation concerns, the Government of India has baned the export of its gum resin (Baillie et al., 2004). The plant contains essential oils such as myrcene, dimyrecene, and polymyrecene, along with bioactive compounds including E- and Z-guggulsterone and guggulsterone I and II. These compounds exhibit diverse pharmacological activities, including anti-inflammatory, anti-arthritic, antimicrobial, antioxidant, anti-malarial, muscle relaxant, and larvicidal effects. They are also reported to be beneficial in the management of rheumatism, hyperlipidemia, and obesity. Guggulsterone E and Z plays a significant role in blood lipid levels. Additionally, flavonoids present in the extract may contribute to the management of hypercholesterolemia, hypertension, obesity, and diabetes. Notably, guggulsterone has shown potential anticancer properties by inducing apoptosis and inhibiting tumor growth, invasion, angiogenesis, and metastasis (Khan *et al.*, 2020). Recognizing the role of oxidative stress in cancer and other diseases, this study focused on antioxidant activity and potential inhibitory effects of guggul extracts on human cervical cancer cells (HeLa cell line).

Materials and Methods

The present study was performed in Department of Veterinary Pharmacology and Toxicology and Animal Biotechnology, Madras Veterinary College, Chennai-07. The ethyl acetate extract of guggul was procured from Chemiloids Life Sciences Pvt. Ltd. and subjected to the initial preliminary phytochemical analysis to know the presence of active compounds in

*Corresponding author mendumounika973@gmail.com

[1]Assistant Professor, Centurion University, Odisha

2 Trainee, Centurion University, Odisha

extract and then these active compounds were quantified. The antioxidant potential of the ethyl acetate extract was evaluated using standard in vitro assays. Subsequently, cytotoxic activity was assessed in the HeLa cell line.

Phytochemical test

Qualitative Phytochemical analysis:

The presence of alkaloids, flavonoids, glycosides, reducing sugars, saponins, tannins and terpenoids were tested following the standard procedures (Shaikh and Patil, 2020).

Quantitative phytochemical analysis

Total flavonoids

The total flavonoid content of the extracts was determined by aluminium chloride colorimetric assay (Kamtekar *et al*., 2014).

Total phenolic content

The total phenolic content of the extracts was determined using the Folin–Ciocalteu method using gallic acid as the standard (Maurya and Singh, 2010) and the results were expressed as mg of gallic acid equivalents (GAE) per gram of extract.

Terpenoids

The terpenoid content of the extract was determined as per the standard method (Malik, 2017) and the yield (%) of total terpenoids contents was measured by using the formula (Wi – Wf /Wi x 100).

***In vitro* antioxidant activity**

DPPH (2, 2-diphenyl-1-picryl hydrazyl) assay

The free radical scavenging activity of plant extracts were measured using DPPH as per the method described by Zahin *et al* (2009. The free radical scavenging activity was calculated using the formula given below

% scavenging activity

$$= \frac{\text{(Control absorbance-Test absorbance)}}{\text{(Control absorbance)}} \times 100$$

Reducing power assay

The reducing power assay of the extract was determined using a method described by Pachaiyappan et al. (2014). Ascorbic acid used as the positive control and the results were expressed as µg ascorbic acid equivalents (AAE) per mg of extract.

***In-vitro* cytotoxicity assay**

MTT (3- [4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay for cell viability and proliferation was carried out according to the method described by Mosmann (1983). HeLa cells were seeded at a concentration of $5x10^4$ cells/well in tissue culture-grade 96 wells flat bottom microplates and treated with different concentration of extracts. The plates were incubated at 37°C in a 5% CO_2 for 24 h. After incubation, the medium containing the extract was removed and a 10 µl of MTT dye (0.5 mg/ml) along with 200 µl of fresh media was added to each well. The plates were further incubated at 37°C in 5% CO_2 atmosphere for 4 hours. Subsequently, 100 µl of DMSO was added to each well to solubilize the purple formazan crystals.The absorbance was measured at 570 nm using an ELISA reader. The IC_{50} was determined by probit analysis.

Cell viability (%) = $O.D\ of\ test / O.D\ of\ control$ X 100

Results and Discussion

In recent years, there has been increasing interest in natural antioxidants owing to safety concerns, favorable public perception, and their potential role in reducing the risk of chronic diseases through the consumption of fruits and vegetables. Excessive generation of free radicals can deplete endogenous antioxidant defenses, alter gene expression, and promote the formation of abnormal proteins. These oxidative processes have been implicated in the pathogenesis of more than one hundred human disorders, including atherosclerosis, arthritis,

ischemia–reperfusion injury in various tissues, central nervous system damage, gastritis, cancer, and AIDS.

Qualitative phytochemical analysis

The ethyl acetate extracts of Commiphora mukul were subjected to qualitative phytochemical analysis and the results are presented in Table I. The extracts showed the presence of coumarin, terpenoid, phenol, alkaloid, flavonoid, tannin, quinine and phytosterol.

Table I: Qualitive phytochemical Analysis of ethyl acetate extract of *Commiphora mukul*

Compound	Ethyl acetate extract
Cyanin (betacyanin)	-
Coumarin	+
Terpenoid	+
Phenol	+
Alkaloid	+
Flavonoid	+
Saponin	-
Tannin	+
Glycosides	_
Protein	_
Phytosterol	+
Quinine	+

+ Present; - Absent

Quantitative Phytochemical Analysis

The total phenol, flavonoid and terpenoid content in the ethyl acetate extract of *Commiphora mukul* were presented in Table II.

The extract exhibited higher flavonoid compared to other phytochemicals, while phenolic content is relatively lower. This trend aligns with the findings of Molole *et al.* (2022) in Commiphora mollis. Phenolic compounds and flavonoids are the major plant constituents known for their antioxidant and free radical scavenging activity. Additionallly, the presence of terpenoids in the extract may contribute to antiviral and antibacterial properties.

Table II. Quantitative phytochemical Analysis of ethyl acetate extract of *Commiphora mukul*

Compound	Ethyl acetate extract
Phenol (mg GAE/ g extract)	9.76 ± 0.00
Flavonoid (mg Catechin equivalent /g extract)	84.54 ± 0.17
Terpenoid (%)	58.16 ± 1.1

Values are expressed as Mean ± S.E(n=3)

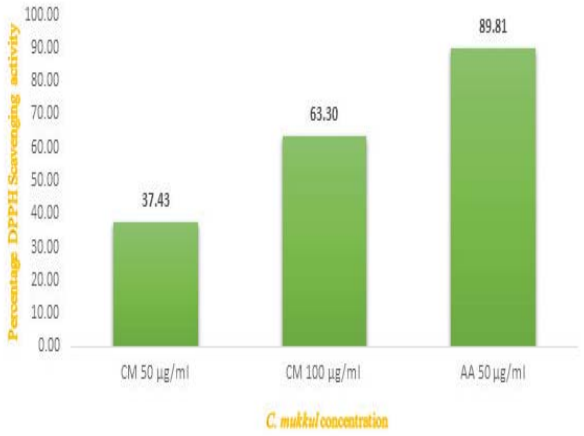
***In Vitro* Antioxidant activity**

Continuous exposure to environment, routine biochemical processes and xenobiotics leads to the persistent generation of reactive oxygen and nitrogen species. These free radicals play a significant role in the pathogenesis of numerous diseases. Antioxidants are widely used to mitigate such oxidative damage. However, concerns over the toxic and mutagenic effects of synthetic antioxidants have driven growing interest in safer, plant-derived natural antioxidants as effective alternatives (Nimse and Pal, 2015).

DPPH (2, 2-diphenyl-1-picryl hydrazyl) Assay

Various in vitro methods are used to evaluate antioxidant activity, though results can vary because the assays are not directly comparable. Among these, free radical scavenging assays are widely employed due to their simplicity, rapidity, and ease of performance (Alam *et al*., 2013). In this study, the proton-donating ability of *Commiphora mukul* extract was assessed using the DPPH assay (Fig. 1), which showed 63.30% scavenging activity at 100 µg/mL. These findings arein agreement with Vani *et al*. (2016), who reported 78.85% activity at a higher concentration.

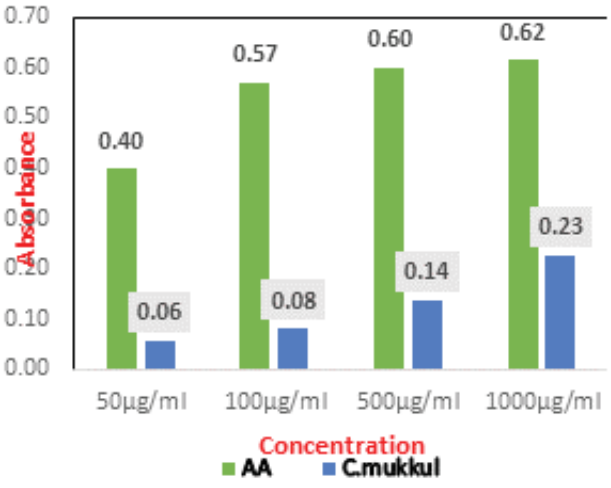
Figure 1: DPPH Scavenging activity of ethyl acetate extract of *Commiphora mukul*



Reducing Power Assay

Reducing power is an important indicator of antioxidant activity, reflecting a compound's ability to donate electrons and neutralize oxidized intermediates during lipid peroxidation, functioning as both primary and secondary antioxidants (Nimse and Pal, 2015). The ethyl acetate extract of Commiphora mukul exhibited a reducing power of 36.49 ± 0.13 µg AAE/mg, which increased proportionally with concentration (Fig. 2). Although lower than that of ascorbic acid, the results indicate significant antioxidant potential of the extract

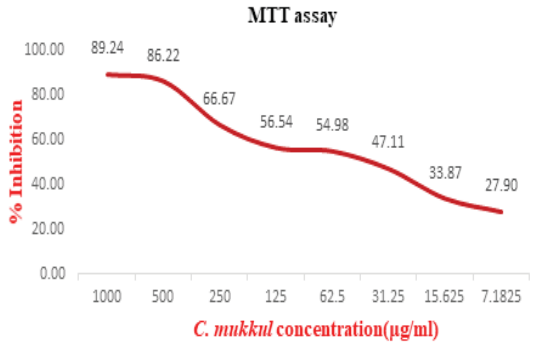
Figure 2: Absorbance in reducing power assay of standard (Ascorbic acid-AA) and *Commiphora mukkul* extract



In vitro MTT Assay

Before the addition of the solubilizing agent, the presence of formazan crystals indicating viable cells was visually confirmed in each wells. The MTT assay results, expressed as % inhibition, were plotted after log transformed concentrations of the plant extract. The resulting plot demonstrated direct correlation between increasing concentration of the plant extract and inhibition of cell viability (Table III and Fig 3).

Fig 3: Cytotoxicity percentage of ethyl acetate extract of Commiphora mukul in HeLa Cell line



The extract exhibited maximum cytotoxicity of 89.24% at 1 mg/ml, with an mean IC50 value of 46.67 µg/ml (range : 37.49 – 57.1). These findings are in accordance with Bharti et al. (2015) who reported an IC50 value of 50 µg/ml in SCC-4 and KB oral cancer cell lines. This suggests the presence of secondary metabolites in the *Commiphora mukul* extract may possess significant anticancer potential, warranting further investigation for the development of novel anticancer agents.

Conclusion

Commiphora mukul is an endangered medicinal plant widely used in traditional medicine and rich in bioactive compounds, including alkaloids, flavonoids, phenols, tannins, phytosterols, and terpenoids. Qualitative analysis confirmed the presence of these constituents, while quantitative results showed higher flavonoid and lower phenol content. Antioxidant assays dem-

onstrated its potential as a natural antioxidant and cytotoxicity findings suggest promising anticancer activity. These results underscore its pharmacological value and emphasize the need for further in vivo studies to validate its therapeutic potential.

Table III: MTT Assay: Cytotoxicity percentage of ethyl acetate extract of *Commiphora mukul* in HeLa Cell line

SI. No.	Commiphora mukul extract Concentration	HeLa Cell line (%)
1	1mg/ml	89.24
2	500µg/ml	86.22
3	250µg/ml	66.67
4	125µg/ml	56.54
5	62.5µg/ml	54.98
6	31.25µg/ml	47.11
7	15.625µg/ml	33.87
8	7.8125µg/ml	27.90

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