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Phytochemical Profile, Antimicrobial, Antidiabetic and Anti-inflammatory Studies of *Gynandropsis pentaphylla* Leaves Extracts

Sunanda Bagewadi^{1,} and Sanjeevkumar Giri^{2*}

^{1,2*}Department of Pharmaceutical Chemistry, Karnataka State Akkamahadevi Women's University, Vijayapura - 586108, Karnataka, India

*Corresponding Author:- Dr. Sanjeevkumar Giri

*Assistant Professor, Department of Pharmaceutical Chemistry, Karnataka State Akkamahadevi Women's University, Vijayapura - 586108, Karnataka, India E-Mail: skgiri2748@gmail.com

Article History	Abstract
Received: 13 August 2023 Revised: 30 October 2023 Accepted: 15 Nov. 2023	Every medicinal plant species found potent for various biological properties. The current research work focused on to characterize and investigate the phytochemical and pharmacological properties of <i>Gynandropsis pentaphylla</i> leaves extracts of acetone, ethanol and ethyl acetate. The leaves of <i>Gynandropsis pentaphylla</i> was procured from agricultural terrain in the villages of North Karnataka, in the month of August to October and authentication done at Department of Botany, Karnataka State Akkamahadevi Women's University, Vijayapura, Karnataka, India. The investigation recognizes the presence of alkaloids in acetone extract, glycosides, flavonoids, steroids, saponins, alkaloids in ethanol extract and steroids, glycosides, flavonoids in ethyl acetate extract. The findings from the extracts demonstrated notable antimicrobial against <i>Staphylococcus aureus, Escherichia coli</i> as bacterial strains and <i>Fusarium</i> sps as fungal strain, antidiabetic by α -amylase assay and anti-inflammatory property by hemolysis assay and found significant in ethanol extract compare to acetone and ethyl acetate extracts, hence indicating that ethanol extract is promising prospects for medicinal applications. The results of this study are expected to generate additional research in the fields of phytochemistry and therapeutic application. <i>Keywords: Gynandropsis pentaphylla, Phytochemical profile, Antimicrobial, Antidiabetic, Anti-inflammatory</i>
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1. Introduction

Indian medicinal plants devoted as "Traditional Medicinal System" due to the practice of Ayurveda from thousands of years to treat various diseases and ailments. Around 80% of people worldwide, according to the World Health Organization, get their medical care from herbal sources. Only 17% of the approximately

2,50,000 species of higher plants in the world have been studied for their biological potential as therapeutic medicine [1, 2].

Medicinal plants possess various source of properties and close to 25% of pharmaceutical properties as medicine. As an illustration, study on the often, used local plant *Glycyrrhiza glabra* led to the discovery of carbenoxolone, the first medication helpful in treating gastrointestinal ulcers. Gefarnate was discovered as a result of research on cabbage. *Digitalis purpurea*, a traditional herbal remedy from Europe, was successful, as British physician William Withering discovered in the 18th century. Cardiac glycosides make the heart contract more forcefully and give it more time to rest in between beats [3, 4]. From foxglove leaves, more than 30 cardiac glycosides, including digitoxin and digoxin, have been discovered in the 20th century. Reserpine, an alkaloid still used today to treat high blood pressure, was first isolated from Rauwolfia root in 1949 by German chemists. Artemisinin is the main biologically active ingredient in treating malaria. Alkaloids from *Catharanthus roseus*, which are employed in chemotherapy for children leukemia and the treatment of Hodgkin's disease, were found thanks to research on traditional plants conducted in the United States. The taxol chemical with anticancer properties was identified in the bark of *Taxus brevifolia* [5-7].

The safety, effectiveness, and side effects of therapeutic plants vary. Flavoring medications are becoming the therapy of choice in Asian nations, especially for the agricultural population. Ancient writings also describe the benefits of herbal remedies for age-related illnesses like dementia, osteoporosis, osteoarthritis, diabetes, immunological and liver disorders, for which there is no modern medicine or only palliative care. They are thought to be more compatible with the human body since they contain chemical components that are essential to the physiological processes of living things [8-10].

Analgesics, antibiotics, and non-steroidal anti-inflammatory medicines are some of the therapy options that are available for wound management. But the bulk of these treatments result in a variety of undesirable side effects. Numerous investigations into the potential of herbal medicines for the treatment of wounds have been conducted in recent years. In comparison to synthetic medications now on the market for the treatment of wounds, these natural therapies have demonstrated their efficacy. Pharmacological studies have revealed that a variety of natural herbs have strong wound-healing properties [11-18].

In this scenario, the present study is to provide a comprehensive analysis of phytochemical profile acetone, ethanol and ethyl acetate extract derived from the leaves of *Gynandropsis pentaphylla* with a particular focus on elucidating their biological evaluation and their potential pharmacological activities.

2. Material and Methods

The reagents, solvents, and chemicals required for this study were used of analytical grade and procured from Hi-media Laboratories Pvt. Ltd., Mumbai and Qualigens Fine Chemicals Pvt. Ltd, Mumbai. The leaves of *Gynandropsis pentaphylla* was collected from agricultural area of North Karnataka, in the month of August to October 2023 and authenticated in Department of Botany, as per the herbarium accession and flora deposits in the Indian medicinal plant directory.

2.1 Preparation of Plant Material

Gynandropsis pentaphylla leaves that had been collected as whole plant were cleaned and rinsed with tap water, then washed with distilled water and sterilized with 70% ethanol. For 10 days in shade condition plant leaves were thoroughly dried in the room temperature. The leaves were mechanically grinded to make it as powder, neatly packed in glass container and stored in refrigerator. Later used for successive Soxhlet extraction with acetone, ethanol and ethyl acetate for 18 hours, and dried at Buchi's rotary vacuum evaporator and stored in refrigerator [19].

2.2 Analysis of Phytochemical Profile

Qualitative phytochemical analysis of the acetone, ethanol and ethyl acetate extract of the leaves was processed to identify the secondary metabolites. The all the extracts of *Gnandropsis pentaphylla* leaves were analysed by defined phytochemical methods as per the Fransworth [20], Harborne [21] and Sharangouda and Patil [22] to explore the metabolites such as steroids & tritepenoids, alkaloids, tannins, flavonoids, glycosides, carbohydrates, fatty acids, saponins, lignins, proteins and amino acids. The extract was suspended in 0.2% Tween 80, dissolved in double distilled water and filtered. The filtrate was used for the phytochemical profile in triplicate to obtain the concurrent value for statistical analysis.

2.3 Antimicrobial Activity Study by Zone of Inhibition Method of *Gynandropsis pentaphylla* Leaves Extracts

2.3.1 Test Microorganisms and Growth Media

The following microorganisms *Staphylococcus aureus*, *Escherichia coli* and fungal strain *Fusarium sp* was chosen based on their clinical and pharmacological importance. The microbial strains obtained from National Collection of Industrial of Microorganisms (NCIM), Pune, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and Czapek-Dox Agar medium (Hi-Media Pvt. Ltd, Mumbai, India), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in nutrient agar plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar plates at 4°C) and the stock cultures were maintained at 4°C.

2.3.2 Minimum Inhibitory Concentration

In vitro detection of minimum inhibitory concentration was examined for *Gynandropsis pentaphylla* leaves extract of Acetone, Ethanol and Ethyl Acetate. Minimum inhibitory concentration of extracts against bacteria and fungi was investigated by the agar diffusion method. The extracts were used for the determination of zone of inhibition or sensitivity, against *Staphylococcus aureus*, *Escherichia coli* as bacterial strains and *Fusarium* as fungal strain.

The nutrient agar and Czapek-dox agar was prepared as per the composition mentioned below (Table 1), autoclaved for 121°C for 15 lbs and cooled and poured on sterilized petri plates and allowed for solidification. After solidification inoculate the bacterial test strain make a lawn streak later make the well and introduce the sample in different plate. Incubate the plates at 37°C for 24 hours for bacteria and at 30°C for 72 hours for fungi. The incubated plates were observed for growth inhibition activities.

Table-1: Nutrient media composition for bacterial and fungal strain studies							
Nutrient agar		Czapek-Dox Agar					
Composition	Concentration (%)	Composition	Concentration (%)				
Peptone	0.5%	Sucrose	3				
Yeast extract	0.3%	Sodium Nitrate	0.2				
Sodium chloride	0.5%	Magnesium Sulphate	0.05				
Agar	1.5%	Potassium Chloride	0.05				
Ph	6.8±0.2	Sodium dihydrogen phosphate	0.1				
		Ferrous sulphate	0.0025				
		Agar	2				
		Ph	5.0±0.2				

2.4 Detection of Antidiabetic Property of Gynandropsis pentaphylla Leaves Extracts

Antidiabetic property of the *Gynandropsis pentaphylla* leaves extracts were determined spectrophotometrically at 540nm, α - amylase causative agent to release glucose by the hydrolysis of starch substrate blocking ability is tested for the samples. Test extracts were treated with α -amylase for a period of time later the reaction mixture is treated with starch to check the efficiency of α -amylase to release glucose, the concentration of glucose liberated is determined at 540nm using Di-nitro salicylic acid and the percentage of anti-diabetic property is determined by the below formula.

Anti-diabetic Property % = <u>Control Absorbance – Sample Absorbance</u> X 100 Control Absorbance

2.5 Detection of Anti-Inflammatory Property of Gynandropsis pentaphylla Leaves Extracts

Anti-inflammatory property of the *Gynandropsis pentaphylla* leaves extracts were determined spectrophotometrically at 560nm, human red blood cell (HRBC) membrane stabilization method using suspension was prepared from the blood of healthy individual, the blood sample collected was mixed with equal volume of aqueous alsever's solution containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride and isosaline, the mixture was centrifuged at 5000rpm for 10 minutes, the supernatant obtained after centrifugation is used as HRBC suspension for the test. The compound to be tested is treated with equal volume of HRBC suspension and incubated at 35°C for 30 minutes, later the test sample mixture is centrifuged at 3000rpm for 5 minutes; absorbance of the obtained supernatant is measured at 560nm. The absorbance of HRBC suspension is taken as control. The percentage of haemolysis and the percentage of protection is calculated using below formula to know the anti-inflammatory property.

Percentage of Heamolysis = <u>Test Absorbance</u> X 100 Control Absorbance

3. Results and Discussion

3.1 Phytochemicals Profile of Gynandropsis pentaphylla Acetone, Ethanol and Ethyl Acetate Extracts

The results of qualitative analysis of phytochemical profile of the all the extract of *G. pentaphylla* leaves are shown in Table 1. It was observed that leaves of acetone extract shown Alkaloids only, in ethanol extract Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Steroids were present in the leaves extract and in ethyl acetate shown Glycosides, Steroids were present and others were absent in the extracts. Similar findings corelate with our studies done on various medicinal plants to report on in vivo and in vitro studies on animal model as well as cell lines [23-32].

Table 1: Phytochemical analysis of *G. pentaphylla* acetone, ethanol and ethyl acetate soxhlet extract shows the presence of the following.

		Soxhlet Extraction of Gynandropsis pentaphylla leaves						
Phytoc	hemical Profile	Acetone	Ethanol	Ethyl Acetate				
1.	Alkaloids	+ ve	+ ve	- ve				
2.	Flavonoids	- ve	+ ve	- ve				
3.	Glycosides	- ve	+ ve	+ ve				
4.	Lignins	- ve	- ve	- ve				
5.	Phenols	- ve	+ ve	- ve				
6.	Steroids	- ve	+ ve	+ ve				
7.	Saponins	- ve	+ ve	- ve				

3.2 Results of Minimum Inhibitory Concentration of *Gynandropsis pentaphylla* Leaves Extracts of Acetone, Ethanol and Ethyl Acetate

In the above table and figures, test results of the antimicrobial activity screening can be observed. The test was performed on the acetone, ethanol and ethyl extracts by taking three organisms, two bacterial strains (*Staphylococcus aureus and Escherichia coli*) and one fungal (*Fusarium sp.*) using six different concentrations (25, 50, 75, 100, 150 and 200) of the sample.

The result shows the extract having significant effect in controlling the growth of both bacterial and fungal strains exhibited 2 mm zone of inhibitions at 150 and 200 µl concentration compare to other concentrations. Similar research findings were reported by many of the researchers on various plants on similar organisms to determine antibacterial and antifungal activities, such as *Azadirachta indica, Syzygium aromaticum, Piper nigrum, Piper roxburghiana, Cinnamomum tamala,* C, *Nelumbo nucifera, Nigella sativa, Withania somnifera, Withania coagulens, Tinopspora cordifolia, Rhus semialata* were enlisted in antimicrobial potential due to their broad-spectrum biological action against various microorganisms [2, 9, 18].

Test Organism	Test Organism Zone of Inhibition in <i>Gynandropsis pentaphylla</i> leaves extracts																	
	Acetone Extract					Ethanol extract					Ethyl Acetate extract							
	Con	centra	tion in	μl			Concentration in µl				Concentration in µl							
	25	50	75	100	150	200	25	50	75	100	150	200	25	50	75	100	150	200
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2mm
Staphylococcus	0	0	0	0	0	0	0	1mm	1	2	1mm	1mm	0	0	0	0	1mm	1mm
aureus									mm	mm								
Fusarium sps	0	0	0	0	1mm	2mm	0	0	0	0	1mm	2mm	0	0	0	0	2mm	3mm

Table 2: Detection of Minimum Inhibitory Concentration of Gynandropsis pentaphylla leaves extracts





Figure 1: Antimicrobial activity of acetone (A Sample), ethanol (E sample) and ethyl acetate (EA Sample) extract of Minimum Inhibitory Concentration of *Gynandropsis pentaphylla* leaves extracts (25, 50, 75, 100, 150 and 200 µl concentration) bacterial strains a) *Staphylococcus aureus* b) *Escherichia coli*, and for fungal strains c) *Fusarium* sps.

3.3 Results of Antidiabetic Property of Gynandropsis pentaphylla Leaves Extracts

The α - amylase is causative agent to release glucose by the hydrolysis of starch substrate when it is blocking the ability when treated with plant extracts. All the three extracts were treated 100% concentration with α amylase for particular time period to react with mixture when treated with starch to check the efficiency of α -amylase during the release of glucose, the concentration of glucose liberated is determined and observed the activity 55% at Ethyl acetate, 1.5% at Ethanol extract and absent at Acetone extract when compared to positive control Di-nitro salicylic acid and this was exhibited percentage of anti-diabetic property of these plant extract. Similar findings were observed with the plant *Adansonia digitata* Linn. ethanol extract at 250 µg/ml exhibiting 71.1 ± 0.02 percent α -amylase inhibitory as potent antidiabetic potential [34]. Bairagi et al., also revealed antihyperglycemic activity in *Artemisia nilagirica* fruits methanol extract on in vitro study by determining serum glucose concentration at 150 mg/kg body weight exhibiting 52.87±0.35 activity compared to standard and control [35].

Extracts	α-amylase inhibition activity (%)
Di-nitro salicylic acid	100%
Acetone Extract	Absent
Ethanol Extract	1.5%
Ethyl Acetate Extract	55%

Table 3: Antidiabetic property of *Gynandropsis pentaphylla* leaves extracts

3.4 Results of Anti-Inflammatory Property of Gynandropsis pentaphylla Leaves Extracts

The plant extracts of all the three were treated with equal volume of HRBC suspension and incubated at 35°C for 30 minutes and observed haemolysis. The results of HRBC suspension is taken as control to determine the percentage of haemolysis and protection to exhibit the anti-inflammatory property. The anti-inflammatory activity of acetone extract was 67% and 33%, ethanol shows absent and in ethyl acetate shows 54.5% and 45.5% of percentage of haemolysis and protection respectively when compare to negative control. Similar findings were reported on in vitro anti-inflammatory activity of green synthesized silver nanoparticles of leaf methanolic extract of *Solanum khasianum* Clarke by inhibition of membrane lysis. Anti-inflammatory activity in edema test induced by carrageenan, there was reduction in paw volume at all concentrations of the alkaloid from chloroform, methanol and water extracts of *Cassia fistula* leaves. As against the standard anti-inflammatory drug, which showed the highest inflammation reduction (76.7%) positive control Diclofenc and in methanol extract showed a significant reduction of inflammation in 55.93% at 200mg/kg inhibition [16, 24, 36].

	Anti-Inflammatory property of Gynandropsis pentaphylla leaves extracts						
Extracts	Percentage of Haemolysis	Percentage of Protection					
Acetone Extract	67%	33%					
Ethanol Extract	Absent	Absent					
Ethyl Acetate Extract	54.5%	45.5%					

Table 4: Anti-Inflammatory property of *Gynandropsis pentaphylla* leaves extracts

4. Conclusion

Analysing the above results and discussion we were conclude the research findings of *Gynandropsis pentaphylla* leaves extracts harbors the phytochemicals which exhibited antimicrobial, antidiabetic and antiinflammatory activities. The anti-inflammatory activity of the plant extracts shows that the further purification of the extracts needs to be taken up to find out the active principles responsible for these pharmacologically important activities. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. The statement has been justified in the current study where the ethanol and ethyl acetate extracts of *G. pentaphylla* showed maximum total antimicrobial, antidiabetic and anti-inflammatory properties compare to the acetone extract. Chromatography and spectroscopy will reveal the more evidence for the further investigation to prove the novelty of the plant studies.

Conflict of Interest

No conflict of interest

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