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Biomedical Potential of *Gynandropis pentaphylla* Leaves Indigenous from Chhattisgarh India

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Abstract

Central India has one of the biggest biodiversity regions in all over Asia, sharing rich medicinal and aromatic plant diversity and their therapeutic purposes represent a secular tradition in different cultures. The genus *Gynandropis* has high medicinal properties among some of the astounding herbs known for enormous potentialities and is also known to be employed for the treatment of cancer in some parts of the world paving a way for replacing the allopathic treatments with the natural ones. The present work has thus been designed to check the antimicrobial potential of the same and identify the respective compound. The antimicrobial profile of hydro-alcohol, aqueous, methanol and ethanol, hexane, chloroform and petroleum ether extract of *Gynandropis pentaphylla* indicated that the methanol and ethanol extract shown significant antibacterial efficacy against pathogens viz., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Brevibacillus brevis* and *Enterococcus faecalis*, *Vibrio cholerae, Aspergillus niger, Candida albicans*, *Schizosaccharomyces* sp. and *S. cerevisiae*. The petroleum ether extract of *Gynandropis pentaphylla* exhibited least activity against test pathogens. The FTIR and HPLC analysis have revealed the presence of Quercetin as the major bioactive metabolite.

Keywords: Antimicrobials, HPLC Analysis, Gynandropis, Phytochemical, TLC Profile

Introduction

In recent times, the modern science and society accept the medicinal values of traditional plants as an alternative of allopathic synthetic health care medication. Presently, the resistance development in microbial communities against the routine drugs used to treat them are the major concern in healthcare sector **(Alviano and Alviano, 2009)**. Plants are the prosperous source of bioactive compounds viz., secondary metabolites i.e., alkaloids, flavonoids, phenols, polyphenols, saponins, steroids and tannins derivatives, that could potentially be utilized as potential antimicrobial agent against pathogenic bacterial strains due to their biological efficacy **(Lisec et al, 2015).** These naturally derived bioactive agents have played significant role in pharmacological sector to construct a variety of health care products e.g antibacterial, anti-tumour and antihepatotoxic drugs **(Gortzi et al, 2007)**. Several herbs, shrubs, trees and climbers have been explored for bioactive agents for verity of medicinal applications in healthcare

industries **(Ezzatzadeh et al,2012)**. The medicinal plants are considered as their promising application to prevent and cure variety of microbial infections **(Ncube et al,2012)**. The active ingredients extracted from medicinal flora by means of organic solvents are more significant **(Mitra and Sur 1997)**. The interface between active secondary metabolites might have induced the remedial properties of medicinal plant extracts **(Jaiswal and Jain, 2017)**. Biological pathways have generated harmful free radicals and superoxide as by-products and these may cause mutation and associated disorders **(Adiguzel et al 2009)**. The secondary metabolites i.e., phenolic acids and flavonoids have an antioxidant nature that might saturate the free radical reactivity to mitigate undesirable effects on human health **(Choi et al 2019).**

Traditional medicinal plants have thus been well recognized as sources of various bioactive compounds. Such established therapeutic efficacies have enabled the pharmaceutical industries to use their crude extracts in manufacturing drugs **(Meskin et al, 2002)**. Modern pharmaceutical companies are now relying on research conducted on medicinal plants serving the dual purpose of identifying potential bioactive therapeutic agents and synthesizing drugs based on their chemical structure **(Unnikrishnan, 2010).** As compared to the enormous potential of earth's plants, only a small fraction has been explored till now **(Chidozie et al 2014).** Hence, more organized efforts are required based on bioassay determination of natural products from medicinal plants.

*Gynandropis pentaphylla (*Syn. *Gynandropis gynandra,* (L) and *Cleome gynandra* (L) family Capparidaceae**)(Chweya and Mnzava, 1997)** is an orphan leafy, herbaceous plant also known as cat whiskers and spider's web in English and *Hur-hur* or *karaila* in Hindi. Plants belonging to this genus are typically C_4 plants having widespread populations in tropical areas of the world **(Van Den Bergh et al, 2014).** In several communities of India and especially Chhattisgarh the plant is cultivated in home gardens. The leaves and young shoots are often consumed as pot herbs adding in soups and stew. Sometimes, the leaves are blanched and dried for preservation also. **(Flyman and Afolayan, 2006).**

G. pentaphylla leaves has been traditionally used in Indian medical practices like Ayurveda for several years. The leaves are still used as disinfectants, for eye wash and earache. The whole plant has been reported to have remarkable antimicrobial, antihelminthic and antifungal properties (**Sridhar et al, 2014).** Recent studies have also demonstrated antiproliferative, antineoplastic and anticancer activities of *G. pentaphylla* on various cell lines **(Rajendran et al, 2014, Pettit et al, 2005, Bala et al, 2010).**

Keeping the above facts in mind, the present study was designed and proceeded accordingly.

Materials and Methods

Sample collection and Processing

The *Gynandropis pentaphylla* plants were collected from Raipur district and final identification was done by Dr. P.K. Joshi, Principal Scientist, Centre of Excellence on MAPs and NTFP, IGKV, Raipur. The leaves of collected plant material were then processed viz., oven dried and pulverized into powder. The extracts were prepared by cold percolation method. The required fraction of grinded powder was soaked in (50% w/v) hydro-alcohol, aqueous methanol and

ethanol, hexane, chloroform and petroleum ether for 72 h. The prepared mixtures were stirred using a sterile glass rod at 24 h interval. The extracts were filtered by Whatmann filter paper no 1**(Dulger and Gonuz, 2004)**. The filtrates were then concentrated in water bath.

Antimicrobial Profile

The antimicrobial profile of each extract was evaluated against a set of Gram positive and negative pathogenic bacteria using Kirby – Bauer method determined by NCCLS standards **(National Committee for Clinical Laboratory Standards, 2002)** and fungi using Poisoned food technique **(Pundir et al, 2010)**. The test pathogens were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India. The following bacterial strains were obtained- *Alcaligens faecalis (MTCC 9780), Brevibacterium brevis (MTCC 3136), Bacillus cereus (MTCC- 1272), Enterococcus faecalis (MTCC- 2729), Staphylococcus aureus (MTCC-3160), Escherichia coli (MTCC- 3221), Klebsiella pneumoniae (MTCC- 9544), Pseudomonas aeruginosa (MTCC- 3163), Salmonella typhi (MTCC- 733)* and *Vibrio cholerae (MTCC-3904).* The fungal strains obtained were as following *Aspergillus niger (MTCC- 478), Candida albicans (MTCC-183), Saccharomyces cerevisiae (MTCC- 170), Schizosaccharomyces japonicus (MTCC- 3061).* The bacterial strains were grown on Soyabean Casein Agar at 37 ^{0}C. The fungal strains were grown on Sabouraud's Dextrose Agar at 25^{0}C. The pre-prepared inoculums of each pathogenic bacterium were spread over the Muller-Hinton agar surface evenly. Each disc (6.0 mm diameter) was impregnated with different plant extract at pre-determined concentration and oven dried at 40^{0}C. The impregnated discs were placed into the Muller-Hinton agar surface aseptically. The bacterial and yeasts plates were incubated at 35±2^{0}C and 28±2^{0}C respectively. The plates were examined at 24 to 48h of interval. The clear zones were measured in millimeter using ruler **(Mathur et al, 2011).** The positive and negative control was taken. The positive control used was Azithromycin (1µg/ml) for bacterial plates and Fluconazole (1 µg/ml) for fungal plates. The tests were done in triplicates.

Phytochemical Profile

Major phytochemical viz., alkaloids, flavonoids, glycosides, reducing sugars, saponin, steroids and tannins were examined **(Kamal, 2014).**

Alkaloids- Pre-warmed 1% HCl (5.0 ml) was mixed with 0.5 g of the plant extract and filtered. 1.0 ml filtrate was blended with Dragendroff's reagent. The appearance of turbidity or precipitation was observed for the presence of alkaloids.

Flavonoids- Methanol was mixed with 0.2 g of the plant extract and heated. After addition of Magnesium metal and little HCl (concentrated), the appearance of red/orange color was observed.

Glycosides- The glacial acetic acid (2.0 ml) consisted of few drops of $FeCl_3$ (1%) mixed with 0.5 g of plant extract. Upon the addition of conc. H_2SO_4, appearance brown ring at the interphase was observed.

Reducing Sugars- Fehling's solutions-I and II (1 ml) was mixed with 2.0 ml of the plant extract and put on boiling water bath for five minutes. The appearance of brick red precipitate was observed for the confirmation of reducing sugars.

Saponin-The distilled water mixed with 0.5 g of the plant extract and shaken rigorously. The appearance of froth when warmed confirmed the saponin.

Steroids- Salkowaski method was used. The chloroform (3.0 ml) was mixed with 0.5 g of plant extract and filtered. The H_2SO_4(concentrated) was added to the filtrate. The appearing of Reddish-brown colored ring was observed for the presence of steroids.

Tannins- Boiling distilled water (10 ml) was mixed with 0.5 g of the plant extract and filtered. Few drops of $FeCl_3$(6.0%) were added. Appearance of dark green color confirmed the tannins.

Separation and purification of bioactive compound from plant extract

Thin Layer Chromatography

The Thin Layer Chromatography analysis was performed on potent plant extract with maximum antimicrobial activity using silica gel bed on a glass plate focusing on detection of steroids, alkaloids, flavonoids and polyphenol groups (Sani et al, 2018). The extracts were loaded on one end of the glass plate. The solvent system used was n-Hexane: Ethyl acetate in the ratio 7:3. For the detection of alkaloids, flavonoids and polyphenols Dragndorff reagent, Sitrobaric reagent and $FeCl_3$ and UV light were used as spraying reagent.

Identification and structure elucidation of purified compounds

Identification of purified compounds was done using the combination high performance liquid chromatography (HPLC) and Fourier transform infrared (FTIR) spectrometry.

High Performance Liquid Chromatography Analysis

HPLC (Shimadzo LC- 2010 HPLC system, Kyoto, Japan) of plant extract was performed by NCS Green Earth Pvt. Ltd., Nagpur, Maharashtra, India. The HPLC outfitted with a Shimadzo LC 2010 UV-VIS detector The C-18 column block (heating-type Shim-pack VP-ODS, 4.6 mm interior diameter × 150 mm long), particle size of 5.0 µm was used. Mobile phase consisted of 50 % acetonitrile and50% Phosphate buffer at a flow rate of 3.0 ml/min at 25°C. Sample volume was 40 µl. The wavelength for maximum absorbance was obtained by UV absorption spectra of the purified plant extract using UV-VIS Spectrophotometer.

FTIR Analysis

The FTIR (Perkin Co., Germany) plant extract was performed by NCS Group, Nagpur, Maharashtra, India. The FTIR spectrum was taken at a range of 4000-400 cm^{-1}using KBr pellet technique.

The result obtained from FTIR spectrometry of purified compound (separated from plant extract) was correlated with literature values.

Statistical Analysis

All the experiments were done in triplicates and standard error mean was calculated. The tabulation of data and statistical analysis was done by MS-Excel 2010.

Results and Discussion

The present investigation was performed to assess the antimicrobial efficacy of secondary metabolites of *Gynandropis pentaphylla*. The isolation and identification of potential purified bioactive compound from plant extract was done using FTIR and HPLC.

Antimicrobial Profile

The antimicrobial profile of hydro-alcohol, aqueous methanol and ethanol, hexane, chloroform and petroleum ether extract of *Gynandropis pentaphylla* was determined **(Table I).** Observations indicated that the methanol extract has shown significant antibacterial efficacy against pathogens viz., *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Enterococcus faecalis*. The non-polar solvents i.e hexane and chloroform- based extract were shown to be effective against *Escherichia coli* and *Enterococcus faecalis* correspondingly whereas antimicrobial efficacy against *Brevibacillus brevis* and *Vibrio cholerae* was slightly lower. The petroleum ether extract of *Gynandropis pentaphylla* was found to exhibited least activity against the test pathogens. Almost every extract of *Gynandropis pentaphylla* exhibited noteworthy activity against *Aspergillus niger*, *Candida albicans*, *Schizosaccharomyces japonicus* and *S. cerevisiae* except petroleum ether-based extract. The methanolic extract exhibited maximum anti-fungal/yeast activity against *Schizosaccharomyces*, *Aspergillus niger* and *Candida albicans* whereas least activity against *S. cerevisiae*.

Table 1: Antimicrobial activity of *Gynandropis pentaphylla* extract against human pathogenic bacteria and fungi/yeast through Disk Diffusion Method.

Bacterial Test Pathogen	**Hydro-alcoholic extract**	**Methanol extract**	**Ethanol extract**	**Hexane extract**	**Chloroform extract**	**Petroleum ether extract**	**Ref. (Azithromycin)**
Alcaligenes faecalis	10.50±0.08	13.83±0.00	ND	ND	9.00±0.28	ND	35±0.06
B. cereus	11.16±0.02	10.12±0.02	10.0±0.14	15.5±0.99	8.00±0.01	6.63±0.05	32.1±0.09
Brevibacillus brevis	18.50±0.02	17.16±0.15	21.00±0.10	7.63±0.11	6.63±0.05	NA	33.0±0.21
Enterococcus faecalis	19.16±0.05	15.00±0.00	24.80±0.09	16.2±0.08	9.50±0.07	NA	29.2±0.18
Staphylococcus aureus	21.83±0.11	21.00±0.10	25.83±0.00	11.00±0.04	8.80±0.07	NA	26.25±0.02

Escherichia coli	19.00±0.00	NA	18.00±0.14	19.0±0.12	16.16±0.01	NA	36.25±0.02
Klebsiella pneumoniae	ND	12.50±0.04	10.67±0.20	NA	NA	NA	30.01±0.09
Pseudomonas aeruginosa	22.83±0.10	23.00±0.07	20.83±0.84	15.83±0.20	11.50±0.30	ND	34.8±0.07
Salmonella typhi	10.0±0.04	11.83±0.00	NA	ND	ND	ND	28.15±0.15
Vibrio cholerae	ND	12.50±0.10	ND	8.50±0.04	ND	ND	26.18±0.20
Fungal/Yeast Test Pathogen	**Hydro-alcoholic extract**	**Methanol extract**	**Ethanol extract**	**Hexane extract**	**Chloroform extract**	**Petroleum ether extract**	**Ref. (Flucanazol)**
Aspergillus niger	13.00±0.80	17.00±0.019	18.20±0.02	12.16±0.27	NA	NA	16.18±0.00
Candida albicans	16.00±0.05	12.00±0.33	10.00±0.09	13.0±6.01	ND	ND	15.00±0.01
Saccharomyces cerevisiae	NA	11.83±0.15	NA	ND	ND	ND	14.58±0.33
Schizosaccharomyces japonicus	11.50±0.15	17.67±0.15	17.0±0.36	8.00±0.11	14.0±0.01	ND	16.00±0.20

Data are multiple of three observations; Values ± standard error; Ref: Reference antibiotic (Azithromycin and Flucanazol); ND: Not detectable, NA-No activity.

Phytochemical Profile

The phytochemical examination of *Gynandropis pentaphylla* extracts showed presence of significant phytochemicals like alkaloids, flavonoids, steroids, saponin and reducing sugars **(Table 2).**

Table 2: Phytochemical screening of leaves extracts of *Gynandropis pentaphylla*

Solvent extracts	**Phytochemical constituents**						
	Alkaloids	**Flavanoids**	**Glycosides**	**Reducing sugars**	**Saponin**	**Steroids**	**Tannins**
Hydro-alcoholic extract	+	+	-	+	+	-	+
Methanol extract	+	+	-	+	+	-	-
Ethanol extract	+	+	-	+	+	-	+

Hexane extract	-	+	-	+	+	+	+
Chloroform extract	-	-	-	+	+	+	+
Petroleum ether extract	-	-	-	+	+	-	+

+: present; -: absent.

TLC Analysis

Thin Layer Chromatography Analysis have provided insights for the detection of various phytochemicals in the potent plant extracts. Flavonoid was observed as the main phytochemical in almost all extracts except for petroleum ether. The detailed analysis is presented in Table III.

Table III: Thin Layer Chromatography Analysis

Solvent System	**Spray Reagent**	**R_f Value**	**Metabolites**
nHexane: Ethyl acetate	UV Light	0.37, 0.60, 0.74	Steroids
	UV light	0.32,0.56, 0.92	Terpenoids
	Dragendorff Reagent	0.53,0.74	Alkaloids
	Sitroboric Acid	0.20, 0.86, 0.90	Flavonoids
	$FeCl_3$	0.34, 0.63	Polyphenols

Identification and structure elucidation of purified compounds

FT-IR spectroscopy examination of potent plant extract revealed that functional viz., aromatic ring structure, keto, alkane, aryl ether, aromatic-OH, secondary alcohols and benzene ring **(Fig. 1).** The detailed analysis is represented in **Table IV**. The potent methanol and ethanol plant extracts were separated by column chromatography and identified as Quercetin by HPLC **(Fig. 2)**.

Figure 1: FTIR spectra of purified active compound from *Gynandropis pentaphylla*

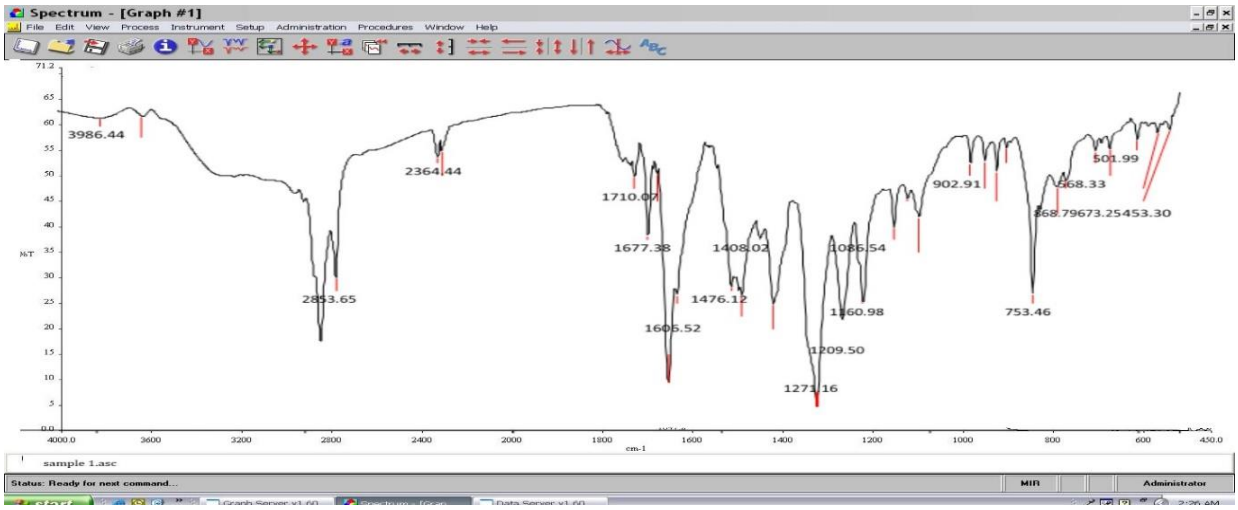
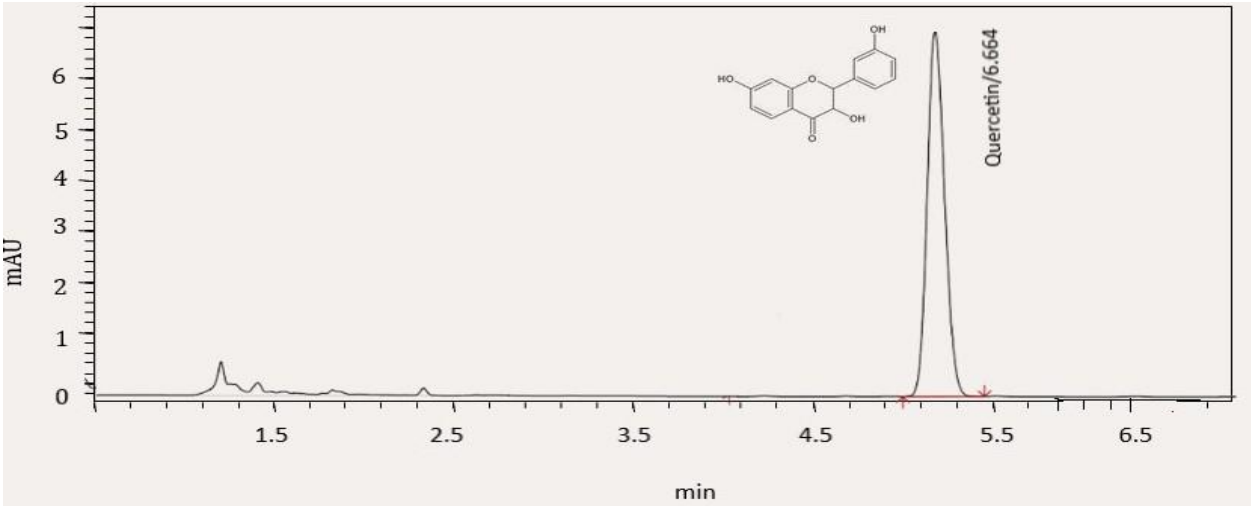


Table 4: FTIR Analysis

Functional Group	FTIR Bands(cm^{-1})	Assignment of Peaks
Aromatic Ring Structure	2925,2853	=C-H Stretching
	2364,2345	Overtone of CH bending possible in side chain structure
	3784	O-H Stretching
Keto group	1710	C=O stretching
Alkane	1476,1452, 1376	C=C ring stretching
	1350-1376	Secondary-OH bending
Aryl Ether	1271	--C—O stretching
Aromatic-OH	1209	With-OH stretching
Secondary Alcohols	1160	With C-O stretching
Benzene Ring	680,694,810	Meta Disubstitutional benzene ring

Figure 2: HPLC chromatogram of purified active compound (Quercetin) of *Gynandropis pentaphylla*



Discussion

Plants belonging to species *Gynandropis* have long been used in various traditional medicinal practices. This information has been thus employed in the present research. Phytochemicals like alkaloids, flavonoids, terpenoids and saponins have been detected in the plant extracts. Similar results were also demonstrated by Borgio et al, 2008 **(Borgio et al, 2008**), whose reports coincided with Ajaiyeoba et al, 2000 **(Ajaiyeoba EO (2000).**

As observed in Table 1 and 2, all the extracts exhibited notable antimicrobial properties with petroleum ether being an exception, in both bacteria and fungi. Similar results were obtained by Thenmozi et al, 2013 **(Thenmozhi et al, 2013).** Activities observed against *B.cereus,*

B.subtilis, S. aureus, P. aeruginosa, E. coli, were in accordance with studies conducted by Kanimathi et al, 2019 **(Kanimathi et al, 2019).**

The flavonoid Quercetin was identified as the purified bioactive compound. This fact is supported by the work done by Kujumgieve, et al 1999 and Erdman, 2007 **(Kujumgiev et al, 1999, Erdman, 2007).**

Conclusion

The *Gynandropis pentaphylla* plants were selected due to their vast pharmacological applications, also they are predominant in Chhattisgarh region of central India. The present research work was focused on the assessment of antimicrobial efficacy of *Gynandropis pentaphylla* followed by the purification and identification of its potential bioactive compound. The advance analytical tools and equipments viz., TLC, HPLC and FTIR were used to obtain valid and authentic database for *Gynandropis pentaphylla.* Quercetin, a flavonoid, was identified as bioactive compound present in *Gynandropis pentaphylla* that act as antimicrobial agent and antioxidant. The present findings could further potentially be contributed as to serve better candidate for the drug development application i.e. antibiotic and antioxidant, in pharmaceutical sector.

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Conflict of interest

The authors declare none.

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