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Research Article

Screening of Functional Groups, DNA Quantification and Determination of Antimicrobial Potency of *Corallocarpus epigaeus* Tubers

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ABSTRACT
This is most probably the first work in isolating genomic DNA from the dried tubers of *Corallocarpus epigaeus* (Ce), which is a medicinal plant, belongs to the family Cucurbitaceae using Cetyl Trimethyl Ammonium Bromide extraction method. Ultraviolet spectroscopy is done for the quantitative determination of DNA isolated. Antimicrobial activity of *Corallocarpus epigaeus* is checked for microbes *Salomonella typhi, Streptococcus luteus, Klebsiella, Aspergillus niger* using the extract prepared from dried tuber with ethanol and benzene and the zone of inhibition was measured. Minimum inhibitory concentration for specific microbes has been determined. Preliminary Phytochemical screening was done to determine the constituents present in the tuber. Fourier transform infrared spectroscopy technique is performed to determine the functional groups present in the tuber. The results revealed the presence of important antimicrobials and also support the continued sustainable use of these plants in traditional systems of medicine.
Keywords: *Corallocarpus epigaeus*, DNA isolation, Antimicrobial activity, phytochemical screening, Ultraviolet spectroscopy, Fourier Transform InfraRed spectroscopy.

INTRODUCTION

The medicinal plant corallocarpus epigeus is commonly known as Agasa garudan in Tamil. It is a medicinal plant traditionally used in siddha and ayurveda as an antivenom for snakebite is not exploited much in modern medicine. The tuber is used in the treatment of chronic rheumatism[1], snakebite, asthma[2], Syphilitic disorder[3]. The tuber extract of C. epigaeus showed higher inhibitory effect than leaf and stem. It may be due to the reason that, the tubers have constant contact with soil, though; they may be infected with soil pathogen. As a result, they produce many antimicrobial substances in response to the infection[4]. The antioxidant and anti-inflammatory activities of Corallocarpus epigaeus rhizomes were evaluated where Ethanol extract inhibited significant anti-inflammatory activity[5]. Pharmacognostical and preliminary phytochemical screening of the root and rhizome of Corallocarpus epigaeus where the presence of alkaloids and flavanoids were confirmed[6]. In vivo screening of corallocarpus epigaeus tuber for its analgesic, anti-pyretic and anti-inflammatory activities was done and analgesic activity, anti-inflammatory property similar to steroidal and non-steroidal agents as well as antipyretic effect were determined[7]. Anti diabetic activity in Corallocarpus epigaeus rhizomes was checked[8]. The above works presented the importance of screening of corallocarpus epigaeus tuber for the identification of potential phytochemicals responsible for medicinal properties.

MATERIALS AND METHODS

Collection of plant material and Preparation of extract: The fresh *Corallocarpus epigaeus* tubers were collected in the fields near Coimbatore. The collected tuber was cleaned and shade dried. The dried tuber was pulverized by an electrical blender. The powder of dried tuber (20 g) was extracted with ethanol, benzene by using Soxhlet apparatus for 24 hrs at room temperature. The extracts were filtered and concentrated at room temperature. After the completion of solvent evaporation, each of these solvent extract were weighed and preserved at 5 ℃ in airtight bottles until further use.

Antimicrobial study: Salomonelle typhi, Streptococcus luteus, Klebsiella, Aspergillus Niger are used to determine the antimicrobial activity of different alcoholic extracts of *Cororallocarpus epigeaus* tuber by disc diffusion method. 24 hour old broth culture of Nutrient media and Potato Dextrose media were prepared and used for antimicrobial plating for bacterial and fungal strains respectively. Consequently, using sterile whatman filter disc of 0.6 cm diameter which were soaked in the solvent ethanol and benzene and the disc soaked with extracts were placed into each agar plates, in aseptic condition. The plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hrs at 37 ℃. The results were recorded by measuring the diameter of inhibitory zone using a transparent meter rule at the end of the 48 hrs.

Preliminary Phytochemical screening of plant extract: The presence of alkaloids, steroids, glycosides, phenols, flavonoids, tannins and terpenoid, in the plant

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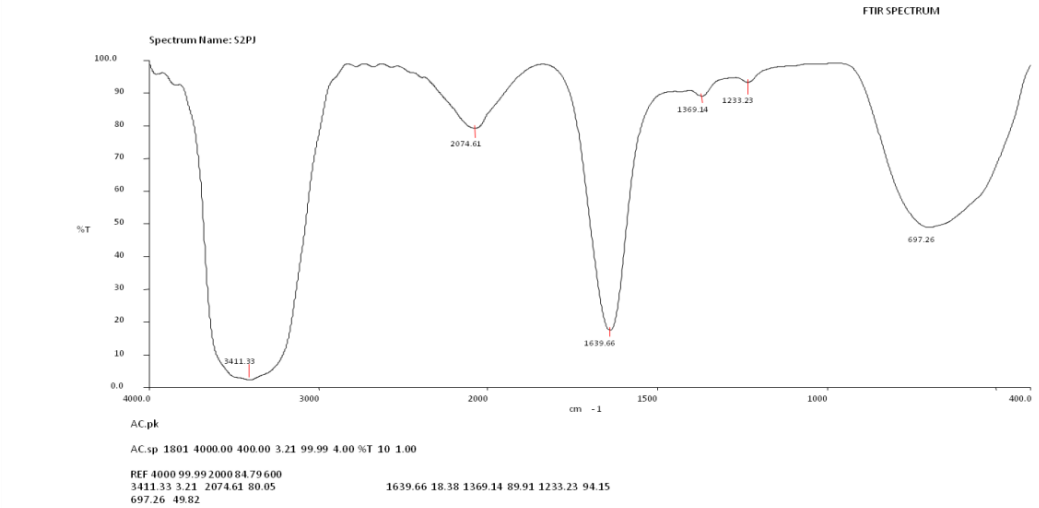

Figure 1: Graphical representation of FTIR Analysis

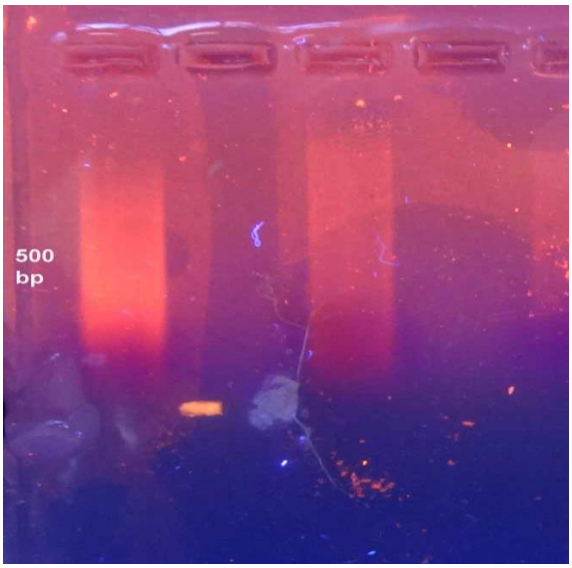

Figure 2: Agarose gel showing the band of genomic DNA of tuber

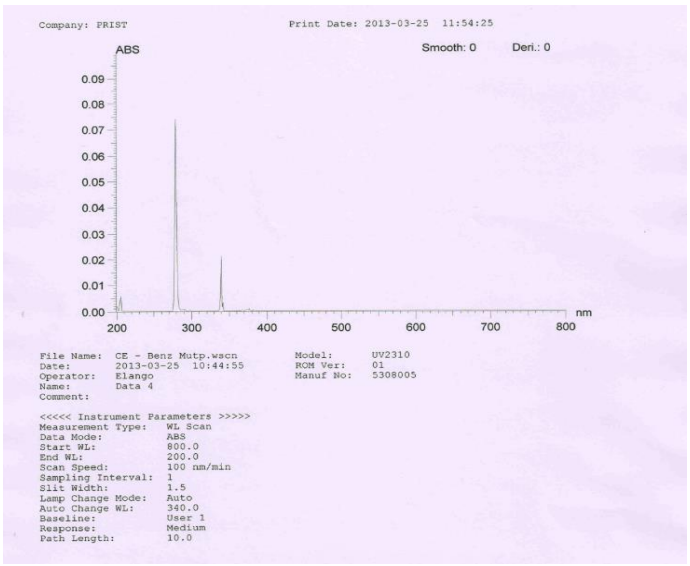

Figure 3: Graphical representation of UV-Spectroscopy Analysis

Corallocarpus epigaeus were analysed by the standard methods of Harborne, 1973[12]

Fourier Transform Infrared Spectroscopy (KBr pellet method): Recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made significant progress in the field of clinical evaluation. . In addition, these techniques also provide molecular-level information allowing investigation of functional groups, bonding types, and molecular conformations .Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. These bands are relatively narrow, easy to resolve, and sensitive to molecular structure, conformation and environment. KBr pellet method is used here. This method required preparation of samples in transparent KBr pellets whose absorbance was measured by the FTIR spectrometer. The concentration of the sample in KBr should be in the range of 0.2% to 1%. About 1/8th of the tuber semisolid sample and about 0.25-0.50 teaspoons of KBr are taken and mixed thoroughly in a mortar while grinding with the pestle. The mixed samples are pressed and placed in the FTIR sample holder (SHIMAZDU 8000 series, 4000-400 cm^{-1}) and spectrum is obtained.

Table 1: Measure of zone of inhibition showing antimicrobial potency against microbes

Microbes	Ethanolic Extract(mm)	Benzene Extract(mm)	Ethanol Solvent(mm)	Benzene Solvent(mm)
S.typhi	13	15	10	10.5
S.luteus	18	16	10	10.5
Klebsiella	12	13	10.5	10
A.niger	13	20	10	10.5

DNA Isolation: Most of the protocols recommend isolation of DNA from fresh tissues, but sometimes the samples collected from remote and rare locations may consist of plant parts in dry or semi-dry condition. These situations necessitate the development of the protocols for isolating DNA from different plant organs, including dry tissues. A rapid DNA isolation protocol that can be used for diverse medicinal and aromatic plants, using dry as well as fresh plant tissues as the starting material [13]. The protocol permitted isolation of DNA from tissues of diverse plant species in fairly good yields, and the isolated DNA proved amenable to PCR amplification and restriction digestion. Samples of plant material were collected, in the form of tuber, from *Corallocarpus epigeus*. The tuber was was dried at 50 ^{0}C for 48 h and the rest was frozen in liquid nitrogen. DNAwas extracted from dry samples. The aliquots (2 ml) of DNA from each sample were loaded on a 0.7% agarose gel to check the quality.

Ultraviolet- Visible Spectroscopy: UV-SPEC technique is performed in order to determine the maximum absorbance of the sample at a particular nanometer and to determine the quantity as well as the quality of the DNA present in the tuber by using the extract prepared by soxhlet through the solvent benzene. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were obtained.

Table 2 Phytochemical screening of plant extract

Compounds	Inference
Alkaloids	+ (Presence)
Steroids	+ (Presence)
Phenols	+ (Presence)
Flavanoids	- (Absence)
Tannins	- (Absence)
Glycosides	- (Absence)
Terpenoids	+ (Presence)
Proteins	+ (Presence)

RESULTS AND DISCUSSION

The present study indicates that the benzene extract of *Corallocarpus epigaeus* showed maximum zone of inhibition for selected Bacteria and Fungi. Phytochemical screening revealed that the ethanolic and benzene extract of tuber *Corallocarpus epigaeus* contains alkaloids, steroids, phenols, terpenoids and proteins. FTIR technique was carried out in order to determine the functional groups present in the tuber by using KBr pellet method. The functional group present in the tuber is listed based on value obtained. The genomic DNA of the sample was isolated and it was analyzed in 0.8% agarose gel which shows clear band of DNA. UV-Spectroscopy revealed the maximum absorbance of the sample at a particular nanometer and to determine the quantity as well as the quality of the DNA present in the tuber. From the spectrum it is found that 0.075 represents the maximum absorbance of the sample taken at 280 nm.

Determination of quality and quantity of DNA in sample by UV SPEC

The quantity and quality of DNA was determined by using the UV-Spectroscopy from the samples of *C.epigaeus* .The OD values that are obtained for the different nanometer for sample is listed in table 4.

Calculation for finding quantity of DNA of sample

Standard formula: 1 OD = 50 µg/ml of DNA

Formula for finding quantity of DNA = *OD at 260 nm* × *conc of DNA at 1 OD*

=11.5 µg/ml of DNA.

Calculation for finding quality of DNA of sample

Formula for finding quality of DNA = *OD at 260 nm* ÷ *OD at 280 nm*

= 0.962343 RNA contamination.

The overall result obtained from our study revealed that the medicinal plant *Corallocarpus epigaeus* has the capability of Antifungal as well as antibacterial activity. The phytochemical analysis revealed that the phytochemicals present in the tuber contributes to antimicrobial activity. Of both the ethanolic and benzene extract of the tuber the extract prepared from benzene exhibited the maximum zone of inhibition. FTIR studies revealed about the functional groups present in the tuber, the most found functional groups are amines, carboxylic acid, and nitrogen compounds. The UVSPEC studies revealed the maximum absorbance of the sample and it is found to be 0.075 at 280 nm. The quality as well as the quantity of DNA was also found by using the photometry data obtained from UV-Spectroscopy for DNA sample of the tuber and the quantity of DNA is 11.5 µg/ml and quality with 0.962343 RNA contaminations. GC-MS and Crystallographic studies will reveal the structure of compounds present in the extract. DNA isolated from dried samples successfully lead to the genome analysis of

Table 4: Photometry Data

DNA Sample	Optical density value	
	At 260 nm	At 280 nm
	0.230	0.239

Table 3: Functional Groups present in Tuber

S.No	Group frequency cm^{-1} of the sample	Functional group assignment
1.	3411.33	Amines N- H stretching vibrations secondary, free; one bond
2.	2074.61	Unsaturated nitrogen compounds, C≡N stretching vibrations isocyanates
3.	1639.66	C-C multiple bond stretching, alkene non conjugated.
4.	1369.14	Carboxylic acids, Carboxylate anion stretching
5.	1233.23	Unsaturated nitrogen compounds, -N_3 stretching vibrations, azides
6.	697.26	C- H bending , Alkene disubstituted

various extinct plant species and their treasure of medicinal properties.

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