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PHYTOCHEMICAL CHARACTERISATION AND BIOLOGICAL ACTIVITIES OF *CAPPARIS DIVARICATA* LEAF EXTRACTS

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Abstract: *Capparis divaricata* is used as antiseptic, in asthma, stomach problems and post-delivery complaints in tribal medicine. The present investigation has been focussed on leaf extracts of *C. divaricate*, screened for phytochemicals, antimicrobial activity and antioxidant activity. Total phenols and total antioxidants were estimated using Folin-ceocaltue reagent and ammonium molybdate reduction assay respectively. GC-MS analysis revealed the presence of 2,6,6- trimethyl-bicyclo[3.1.1]hept-3-ylamine,1,6;2,3-dianhydro-4-deoxy-β-d-lyxo-hexopyranose, Heptanal, Oleic acid, Octanol, 3-nonen-2-ol, 1,3-cyclohexanediol,cis, Z,z,z-1,4,6,9-nona decatetraene, 5,8,11,14-eicosatetraenoic acid methyl ester, (all-z)-, Bisnorallocholanic acid. 1,2-pentadiene 4-methoxy-4-methyl o, Glutaraldehyde, naphthalene propanol, *a*-thyldecahydro-5-(hydroxymethyl)- *a*, 5, 8a-trimethyl-2-methyl. Free-radical scavenging activity was correlated with total phenolic content and total antioxidants. Aqueous and alcoholic leaf extracts were effective on *B. Subtilis* and *K. pneumonia* respectively. The results support the use of plant in the traditional medicines might be useful in various medicaments after thorough in vivo studies.

Index Terms - Capparis divaricata, Heptanal, Oleic acid, Octanol, 3-nonen-2-ol, 1,3-cyclohexanediol, Antibacterial activity, antioxidant potential.

I. INTRODUCTION

India is one of the 17 mega biodiversity centers having 49,000 plant species; its diversity is unmatched due to the 16 different agroclimatic zones, 10 vegetative zones, and 15 biotic provinces [1]. In India, there are about 1500 - 2000 species of codified knowledge, used in traditional medicines like Ayurveda, Unani and Siddha and 3000 species are summarised in ethnomedicine. *Capparis divaricata* is commonly known as spreading keeper. *C. divaricata* is a small tree with spreading branches. Bark is rough brown. Leaves are elliptic, lanceolate, obtuse, mucronate 1-2-inch-long, 0.25-1 inch wide, thick leathery.

Capparis divaricata leaf juice with milk is used in infertility, stomach problems and as analgesic aphrodisiac, diuretic, antiulcer [2]. Leaves are used as anthelmintic, analgesic and aphrodisiac [3]. Madhusudhan Reddy et al. 2019). Evaluation of the locomotor and diuretic activities of ethanolic extract of leaves of *C. divaricata* resulted in significant locomotors and diuretic activity [4]. Evaluation of analgesic activity of ethanolic leaf extract of *C. divaricata* showed significant activity at the dosage of 250 mg/kg [5]. and antipyretic activity [6]. A study of anticancer activity of C. *divaricata* leaves revealed that Chloroform and ethyl acetate extracts

showed significant cytotoxic activity by MTT assay [7]. GC-MS studies of *C. divaricata* revealed the presence of various bio-active compounds [8].

II. MATERIALS AND METHODS

2.1 Collection of Material

The leaves of *C. divaricata* were collected from Ardhagiri hills of Chittoor District. The herbarium specimens were prepared as per the method of Jain and Rao (1983) and deposited in the Department of Botany, S.V. University Tirupati. Provisional identification of voucher specimen (MHL 314) was done by Prof. N. Yasodamma, Department of Botany, S. V. University, Tirupati with the help of Flora of the presidency of madras, Flora of Andhra Pradesh and Flora of Chittoor district. Identification is confirmed after comparing authentic specimens in Herbarium, Department of Botany, S. V. University, Tirupati.

2.2 Preparation of the Extract

C. divaricata leaves were collected and washed with water, shade dried and made into coarse powder which was sieved with 40 mesh sieve to get uniform particle size. A weighed quantity (50gm) of powder was weighed and immersed in 500 ml of distilled ethanol and water respectively for 24 h and the extracts were filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness in a water bath at 50°C. The extracts were stored in screw capped glass bottles in the refrigerator for further use [9].

Qualitative analysis of Phytochemicals

The above obtained extracts were used for Preliminary phytochemical screening of secondary metabolites namely alkaloids, flavonoids, phenols, glycosides, tannins, steroids, lignins, saponins, terpenoids and anthocyanidins etc, was done by the standard procedures prescribed by Gibbs and Kokate et al. [10,11].

2.3 Phytochemical Studies Using Gc-Ms Analysis

Five mg of each extract was taken and dissolved in methanol in a sterile clean test tube. The sample solution was filtered using 0.2 µm nylon membrane and the filtered sample solution was injected into the column for running GC-MS. The analysis was carried out on a Perkin-Elmer workstation, with model Clarus 680 GC coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, DE). Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m x 0.25 mm width film depth of 250 µm capillary tube was used under the following condition. The instrument has an oven with an initial temperature of 60 °C for 2 min and a ramp program which elevates from 10 °C/min up to 300 °C, further 6 min isothermal hold. Helium (He) carrier gas was used, with flow rate split ratios of 10:1. One µl volume of samples were injected and the temperature of the injector was maintained to 260 °C. The mass detector conditions were as follows. Transfer line temperature was 240 °C and ion source temperature was 240 °C. The ionization mode electron impact was at 70 eV and a scan time of 0.2 sec with a scan interval of 0.1 sec. The fragments analyzed were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library. An individual component was recognized with typical mass spectra from National Institute of Standards and Technology (NIST-2008) libraries which is inbuilt by the software of the GC-MS system (TurboMass ver 5.4.2) and literature data. The individual phytochemicals present in the crude extract were separated by the gas chromatography column. An individual compound separated by GC enters the Mass Spectrum (MS) and gets ionized. The MS ionizing spectrum was recorded and compared to the MS spectrum of known compounds in the NIST library. Each compound was compared with a percentage score of reverse and forward spectrum. The MS spectrum displays the molecular weight of individual molecules accurately.

2.4 Quantification of Polyphenols

Total phenolic content was quantified using the Folin–Ciocalteu assay. (Lister and Wilson 2001), This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically. The Folin–Ciocalteu assay is based on an electron transfer, which gives reduced capacity expressed as phenolic content. 10 µl (100 µg) of each extract was taken and added to 100 µl of Folin–Ciocalteu reagent and 300 µl of 20% aqueous sodium carbonate solution and then the reaction mixture was made up to 1 ml. The reaction mixture was incubated in dark for 1 h and the absorbance was recorded at 725 nm. The total phenolic content in each extract was calculated from the calibration curve as µg gallic acid equivalents. The graph was

plotted by taking the concentration of gallic acid in the X-axis and optical density on the Y-axis. All the experiments were performed in triplicate [12].

2.5 Total Antioxidant Capacity

100 µl of each plant extract was added with 1 ml of reagent solution (0.6 M Sulfuric acid, 28 mM sodium phosphate and 4 m M ammonium molybdate) and heated up to 95 °C for 90 min in water. The cooled extracts were tested for the blue color and measured the intensity at 695 nm against a blank. The same procedure is followed and the standard graph using ascorbic acid and compared with the plant extracts and the total antioxidant capacity is expressed as equivalents of ascorbic acid. The calibration curve was prepared using different concentrations of ascorbic acid i. e 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg. The graph was plotted by taking the concentration of ascorbic acid in the X-axis and optical density on the Y-axis. All the experiments were performed in triplicate [13].

2.6 DPPH (2, 2- Diphenyl-1-picrylhydrazyl) free radical scavenging method.

The DPPH method was developed by Blois in 1958 to determine the antioxidant activity using a stable free radical α, α-diphenyl-β-picryl hydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, $M = 394.33$). The assay is based on the measurement of free radical scavenging capacity of antioxidants towards it. DPPH is characterized as a stable free radical conferred by its spare electron over the molecule as a whole and exhibits paramagnetism. So that DPPH does not diminish like others. The delocalisation of electrons gives rise to a deep violet color, with a strong absorption band at around 520 nm. The purple chromogen of DPPH is reduced into a stable, pale yellow diamagnetic molecule by antioxidant or reducing agent by accepting an electron or hydrogen. The activity is expressed as inhibitory concentration $IC_{50,}$ which is the amount of the anti-oxidant necessary to decrease by 50% the initial DPPH concentration. The lowest the DPPH $IC_{50,}$ the heist is the antiradical efficiency [14].

2.7 Antimicrobial Activity – Disc Diffusion Method [15].

The selected bacterial species were grown aseptically on nutrient agar medium was prepared by using the beef extract(5g), Sodium chloride(3g), Peptone (5g), and 20% agar dissolved in distilled water in 1000 ml. and the pH is adjusted to 6.5. The nutrient medium was sterilized using the autoclave at 121^0C and 15 lb pressure and stored for the further usage. All the practices in the laboratory such as preparation of media, culturing of bacterial strains were carried out under the aseptic conditions on the laminar air flow cabinet with proper surface sterilents. Bacteria (Table. 2) were procured from the Department of Microbiology, S.V. University and SVIMS, Tirupati. These were further maintained on nutrient agar slants at 4ºC until further use. *Bacillus subtilis*-ATCC-441, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* - ATCC-741, *Staphylococcus aureus* - ATCC-737 were used in antibacterial assay. The antibacterial assay was conducted using control, standard and test sample discs of various concentrations of extracts on the pre-seeded petri plates with respective bacterial strains. These petri plates were incubated for 24 hours at 35^0C. The results were observed for the inhibition zone around the paper discs are measured using a metric scale in mm. The experiments were repeated for two times and average was taken.

III. RESULTS

3.1 Qualitative analysis of Phytochemicals

The preliminary screening of secondary metabolites revealed the presence of 11 phytoconstituents as Alkaloids, steroids, lignins, flavonoids, phenols, terpenoids, tannins, anthocynidins, cardiac glycosides and saponins reducing sugars.

3.2 Identification of phytoconstituents from the Methanol fractions through GC-MS studies:

GC-MS chromatogram of *C. divaricata* leaf extract revealed 13 peaks (Fig.6)indicating 13 phytochemical constituents. The major compounds identified with theirretention time, molecular formula, molecular weight and peak area are presented are 2,6,6-trimethyl-bicyclo[3.1.1]hept-3-ylamine,1,6;2,3-dianhydro-4-deoxy-β-d-lyxo-hexopyranose, Heptanal, Oleic acid, Octanol, 3-nonen-2-ol, 1,3-cyclohexanediol,cis, Z,z,z-1,4,6,9-nona decatetraene, 5,8,11,14-eicosatetraenoic acid methyl ester, (all-z)-, Bisnorallocholanic acid.1,2-pentadiene 4-methoxy-4-methyl o, Glutaraldehyde, naphthalene propanol *α*-thyldecahydro-5-(hydroxymethyl)- *α*, 5, 8a-trimethyl-2-methyl (Table.1).

3.3 Total Phenolic Content:

The alcoholic extract showed high phenolic content of 265 mg/g dry wt. than the aqueous extract (42 mg/g dry wt). (Table 2.).

3.4 Total Antioxidants:

Highest total antioxidants equivalents of ascorbic acid (vit.C) was observed in alcoholic extract (14.4 mg/g dry weight) than aqueous extract (5.8 mg/g dry weight) and the (Table 2.).

3.5 Antioxidant activity

DPPH Scavenging Activity: *C. divaricata*, aqueous and alcoholic extracts of leaf showed 50% scavenging at 80 µg and 90 µg respectively (Table 2.)

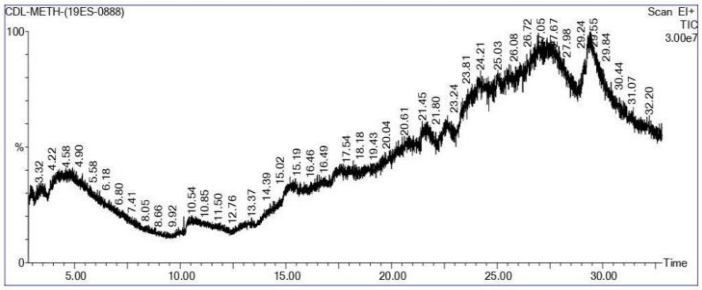
Table 1: GCMS Analysis of *C. divaricata* Methanolic leaf extract

SNo	RT	Name of the compound	Biological Activity
1	22.621	Glutaraldehyde	-
2	23.757	1,6;2,3-dianhydro-4-deoxy-.β.-d-lyxo-hexopyranose	Antidote, anticancer, Depressent, β- Galactosidase-Inhibitor,
3	24.492	Heptanal	
4	24.807	Oleic acid	Acidifier, acidulate, inhibits production of uric acid, arachidonic acid inhibitor
5	25.032	2,6,6-trimethyl-bicyclo[3.1.1]hept-3- yl amine	-
6	25.453	Octanal	antioxidant, antimicrobial, anti-inflammatory
7	26.078	3-nonyn-2-ol	Oligosaccharide provider
8	26.398	1,3-cyclohexanediol, cis	
9	26.948	Z,z,z-1,4,6,9-nonadecatetraene	Increase zink Bioavailability
10	27.503	5,8,11,14-eicosatetraenoicacid, methyl ester, (all-z)-	Catechol-O- Methyltransferase- Inhibitor, Acidifier, Acidulant
11	28.329	1,2-pentadiene, 4-methoxy-4-methylo	Antimicrobial-
12	29.154	1-aphthalenepropanol, *a*.-thyldecahydro-5-(hydroxymethyl)- *a* 5, 8a-trimethyl-2-methyl	*a*-Glucosidase-Inhibitor, *a*-Reductase-Inhibitor,TNF-*a*-Inhibitor
13	29.409	Bisnorallocholanic acid(Steroid with COOH group)	Acidifier, acidulant, inhibits production of uric acid, arachidonic acid inhibitor

Fig 2. GCMS analysis of Methanolic leaf extract

Qualitative Report

File: C:\TurboMass\2019.PRO\Data\CDL-METH-(19ES-0888).raw
Acquired: 17-Jul-19 06:49:10 PM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP
Sample ID: CDL-METH-(19ES-0888)
Printed: 07-Aug-19 10:45 AM
Page 1 of 1
Vial Number: 45



#	RT	Scan	Height	Area	Area %	Norm %
1	23.757	4190	2,374,048	250,534.3	3.623	8.31
2	24.492	4337	3,124,623	258,945.1	3.744	8.59
3	24.807	4400	2,878,288	450,546.2	6.515	14.95
4	25.032	4445	2,831,138	295,074.9	4.267	9.79
5	25.453	4529	3,198,292	262,341.1	3.794	8.70
6	26.078	4654	3,634,234	593,785.6	8.586	19.70
7	26.398	4718	3,401,001	220,777.6	3.193	7.32
8	26.948	4828	3,885,679	351,190.8	5.078	11.65
9	27.503	4939	2,928,038	458,451.6	6.629	15.21
10	28.329	5104	2,257,917	252,830.2	3.656	8.39
11	29.154	5269	4,278,004	506,558.8	7.325	16.80
12	29.409	5320	7,521,474	3,014,467.2	43.590	100.00

Table 2. Total Phenols, Total antioxidants and Free radical scavenging activity

Extract	Total Phenols (mg/g wt)	Total antioxidants (mg/g wt)	Free Radical Scavenging activity (IC_{50} values in µg) DPPH
Aqueous extract	42±01	5.8	80 ±03
Alcoholic Extract	265±04	14.4	90 ±05

Fig 4a: DPPH Standard Graph with Ascorbic acid (Vitamin C)

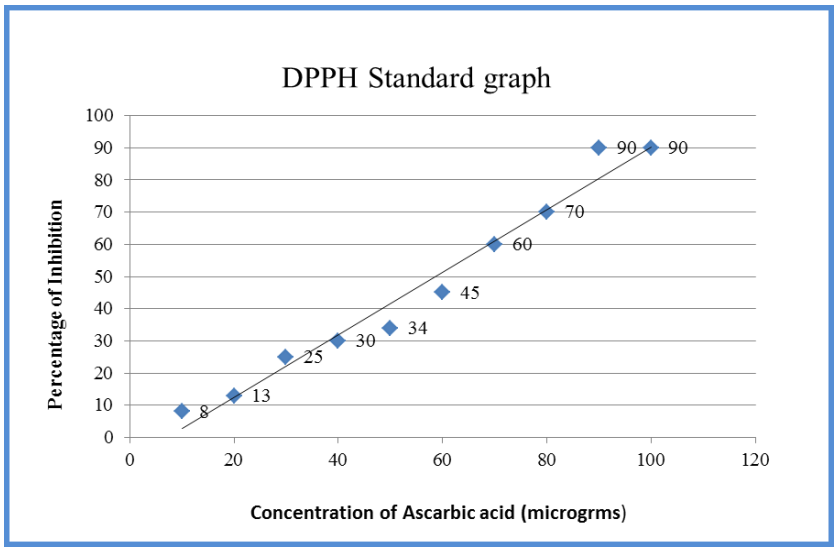
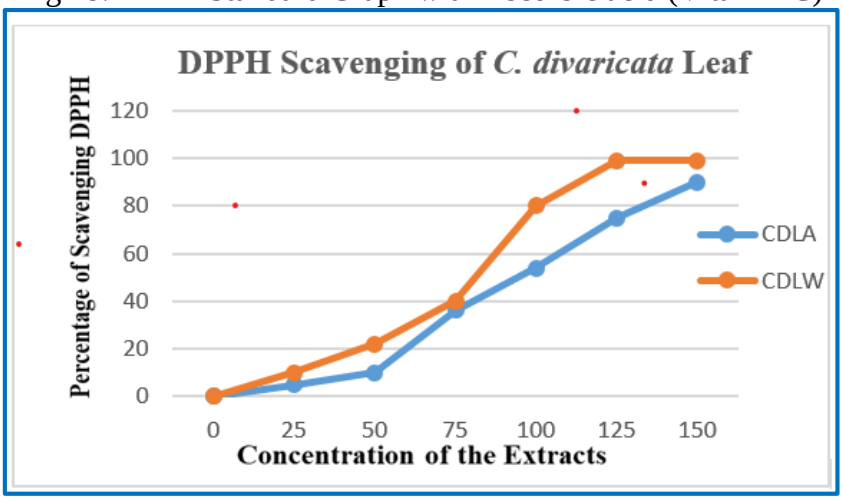


Fig 4b: DPPH Standard Graph with Ascorbic acid (Vitamin C)



3.5 Antibacterial Activity

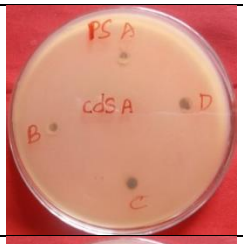
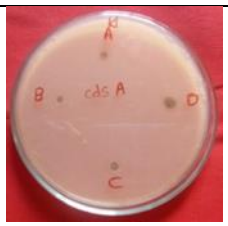
Aqueous leaf extract was effective on *B. Subtilis*, *S. dysenteriae* and *P. aeruginosa*. Ethanolic leaf extract was effective on *K. pneumonia*. (Table. 5 with photographic representation of zones of Inhibition on bacterial strains).

Table 3. Antibacterial activity *C. divaricata* (Zone of Inhibition in mm) leaf extracts

S. No	Microorganism	Ethanol Extract (µg/disc)				Aqueous extract (µg/disc)				AB
		25	50	75	100	25	50	75	100	100
1	*B. subtilis*	-	-	-	-	6± 0.	8± 0.03	9± 0.06	10± 0.03	22
2	*K. pneumoniae*	-	6± 0.20	8± 0.30	10± 0.00	-	-	-	-	16
3	*P. aeruginosa*	-	-	-	-	6± 0.8	6± 0.3	8± 0.	8± 0.28	22
4	*S. decenteriae*	-	-	T	6.4± 0.28	-	T	6.8 ±0.06	8.4 ±0.03	20

T: Trace; AB: Standard antibiotic Amoxicillin

Table 4. Antibacterial activity *C. divaricata* leaf extracts

Name of the organism	*B. subtilis*	*K. pneumoniae*	*P. aeruginosa*	*S. decenteriae*
Aqueous extract				
Alcoholic extract				

IV DISCUSSION

The present investigation has focused on chemical constituents and biological properties revealed potential applications in the field of pharmacology which help in developing the novel medicaments. Evaluation of antimicrobial properties of *Juglans regia.* tree bark revealed 1,6;2,3-dianhydro-4-deoxy-β-d-lyxo - hexopyranose has Antimicrobial activity [16] . Heptanal, Oleic acid acts as an Anticancer agent and reduces cholesterol [17]. A study on biological activities of decanal, linalool, and octanal from sweet orange oil revealed that Octanal an aromatic aldehyde, has antioxidant, antimicrobial, anti-inflammatory activities and it showed cytotoxicity against Hela cells [18]. 5, 8, 11, 14-eicosatetraenoic acid methyl ester, (all-z)- is an important constituent in cell membrane which maintains fluidity and flexibility and necessary for the function of nervous system, skeletal system and immune system. [19]. Bisnorallo Cholanic acid is the major compound which acts as Acidifier, acidulant, inhibits production of uric acid, arachidonic acid inhibitor, increases zinc bioavailability [20].

Similar activities were found in *Capparis divaricata* in which highest scavenging activity was observed in aqueous extract with IC_{50} Value 80 µg/ml. Methanolic stem extract showed least IC_{50} value of 31.83 µg/ml. (Table 2). The selected medicinal plant *C. divaricata* have been used as antiseptic, in post-delivery complaints, for infertility, stomach problems, pain healing, reducing bacterial infections, prevention of cancer, arthritis and as analgesic, diuretic, antiulcer. These medicinal properties are proved that it may be due to the presence of 11 secondary metabolites and 36 bioactive phytoconstituents. Presence of Phenols, antioxidants and bioactive phytochemicals like Pterin 6 carboxylic acid, 1,3-bis-t-butylperoxy-phthalan, (2S,3S) -(-)-3-propyloxirane methanol, Phloroglucinol, S (-)-cathinone, n-acetylo, l-gala-l-ido-octose might be responsible for the antioxidant, analgesic, diuretic, antiulcer and anticancer properties. Propanamide,2-hydroxy, α-methyl-1-adamantane methylamine, 2-(Aminooxy) pentanoic acid are responsible for antimicrobial and pain healing properties. Antibacterial study of C. divaricata demonstrated that aqueous and ethanolic leaf extracts are potent to cure blood stream infections, Urinary and gastrointestinal tract infections, fever, cough, branchial and skin infections. The free radical scavenging effects and antioxidant studies were attributed to protect the biological molecules or membranes from the attack of charged free radicals which may affect the integrity of tissues. Though the significant results are shown on inhibition of the tested bacteria using in vitro studies and require further evaluation using the in vivo system in addition to detailed characterization of the active principle to develop as phyto-antibiotics from these plants. The thorough review and investigation revealed a wide array of medicinal properties, biological activities such as antimicrobial and antioxidant effects. Further research is required to investigate the toxicity of the extracts, dose fixation and evaluation of active principle for developing the novel phytomedicines which are believed to be safe and cost effective.

V. Conclusion

The present investigation on *C. divaricata* leaf extracts revealed that the plant material has potential curing capabilities of tribal (ethnic or traditional) medicinal claims. Hence it has supported the rational information based on the various phytochemical analysis as well as antioxidant and anti-bacterial studies. The plant has been beneficial medicament after careful in vivo research studies.

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