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Phytochemical Contituents Disclosure and Biological Activities of *Capparis Divaricata* Stem Bark Extract

Hemalatha M.[1], Lepakshi Md. Bhakshu[2], Yasodamma N.[3]

1, Department of Botany, SVCR. Government Degree College, Palamaner, 517408. Chittoor (Dt.), Andhra Pradesh, India

[2]Dr. YSR Government Degree College, Vedurukuppam, Chittoor (Dt.), 517 569 Andhra Pradesh India
Corresponding Author Email: *lmbhakshu[at]gmail.com*

[3]Department of Botany, S.V. University, Tirupati, 517 501 Andhra Pradesh, India

Abstract: ***Capparis divaricata is used as antiseptic, in asthma, stomach problems and post-delivery complaints in tribal medicine. C. divaricata stem bark extract was 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 Pterin-6-carboxylic acid, 1,3-bis-t-butylperoxy phthalan, Phloroglucinol, 2,6-lutidine 3,5-dichloro-4-dodecylthio-Propanedioicacid, propyl, (2s,3s)- (-)-3-propnolyloxiranemethanol, Propanamide, 2-hydroxy, 1-adamantanemethylamine, α-methyl, Ethylamine,2-(adamantan-1-yl)-methyl-, Ala-β-ala, trimethylsilyl ester, Urea, heptyl-. Free radical scavenging activity was correlated with total phenolic content and total antioxidants. Aqueous and alcoholic leaf extracts were effective on Schizella decenteriae***

Keywords: *Capparis divaricata*, Pterin-6-carboxylic acid, (2s, 3s)- (-)-3-propnolyloxirane methanol, Propanamide, 2-hydroxy, 1-adamantanemethylamine, α-methyl, Ala-β-ala, trimethylsilyl ester.

1. Introduction

The presence of numerous plant species on Earth is considered a significant natural resource and a treasure in the field of medicine, offering solutions to various human ailments. The use of different parts of plants for treating diseases is referred to as phytomedicine or phytotherapy. The Charaka Samhita, a text from around 1000 B.C., listed over 340 plants and plant-based medications. Traditional knowledge on plant use in ancient cultures, such as in herbal medicine from China, Japan, Korea, and India, continues to influence current healthcare (Samy et al., 2008). The development of drugs from higher plants was proposed by various traditional medical systems (ethno-medicine) for drug discovery (Fabricant and Farnsworth, 2001). Herbal medicines are classified into first generation and second generation. First generation uses simple plant parts and extraction of active compounds. Second generation involves isolating and refining active components through advanced techniques (Iwu et al., 1999). Secondary metabolites such as alkaloids, flavonoids, phenolics, steroids, tannins, terpenoids, saponins, and glycosides are key in both generations. These secondary metabolites serve as protective agents against pathogens, UV radiation, and free radicals, along with antioxidant effects (Tahara, 2007; Jain et al., 2019). Many plant-derived compounds with antioxidant properties help combat oxidative stress-related conditions including anti-inflammatory, anti-aging, anti-atherosclerosis, anti-arthritis, anti-cancer, cardiovascular diseases, diabetes, obesity, and neurodegenerative disorders (Zhang et al., 2015). Natural plant extracts, essential oils, and their components are used in pharmaceuticals, alternative medicine, and natural therapies. Medicinal plants are also recognized as a source of novel antibiotics (Tyagi et al., 2016).

Figure 1: Habit of *C. divaricata* in its natural habitat

Capparis divaricata Lam (Fig. 1) is also called spreading keeper. It is a small tree with branches that spread out. The bark is rough and brown. The leaves are shaped like an ellipse or a spear, with a blunt tip. They are 1 to 2 inches long and 0.25 to 1 inch wide, and they are thick and leathery. The flowers are light green or creamy yellow, about 2 to 2.5 cm across. They grow either alone or in groups of 2 to 3. There are four sepals that are oval in shape. The petals are long and rounded at the tip, and they feel soft and velvety. There are many stamens that are long and thread-like. The gynophore is about 2 cm long. The fruit is round, about 4x4 cm, has 5 to 6 ridges, and has small bumps. The fruit stalk ends in a point. There are many seeds inside. (Gamble, 1967). *C. divaricata* is used in traditional medicine as an antiseptic and for treating asthma and problems after childbirth. (Sandeeep et al., 2011). The bark paste of *C. divaricata* is used to treat dysentery and stomach issues. (Gunasekaran and Balasubramanian, 2012).

2. Materials and Methods

2.1 Collection of Material

C. divaricata leaves 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 stem bark was 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 (Harborne 1999)

2.3 Phytochemical Studies

2.3.1 Preliminary Secondary metabolite Screening

The above obtained extracts were used for Preliminary phytochemical screening of nearly 10 secondary metabolites namely alkaloids, flavonoids, phenols, glycosides, tannins, steroids, lignins, saponins, terpenoids and anthocyanidins was done by the standard procedures prescribed by Gibbs (1974); Kokate *et al.* (2007).

Alkaloids

To the plant extract chloroform was added and the residue obtained was digested with 1% HCl. The acidic solution was taken and made into two parts. 2 ml of Mayer's reagent were added to the first part and 2 ml of Wagner's reagent was added to the second part.

a) **Mayer's reagent test:** 1.3 g of mercuric chloride and 5 g of potassium iodide were dissolved separately in 60 ml and 10 ml of double distilled water then both the solutions were mixed and diluted to 100 ml. Appearance of precipitation and turbidity shows the presence of alkaloids
b) **Wagner's reagent test:** 2 g of Potassium Iodide and 1.27 g of Iodine were dissolved in distilled water and make up to 100 ml distilled water. Appearance of yellowish white precipitate shows the presence of alkaloids.

Flavonoids

a) **Shinoda's test**: 2 ml of the extract were taken and added a few drops of Conc. HCl and then small pieces of magnesium ribbons were added. Pinkish red color was developed indicates the presence of Flavonoids
b) **Ferric chloride test:** To 2–3 ml of the extract, few drops of Ferric chloride solution were added. Production of black color indicates the presence of flavonoids.

Phenolic compounds:

Ferric chloride test: To 2–3 ml of plant extract 1 or 2 drops of 1% ferric chloride solution was added, appearance of intense blue color denotes the presence of phenols.

Test for cardiac glycosides:

a) **Keller-Kiliani test:** 5 ml of the extract is added with glacial acetic acid and 2 drops of ferric chloride and transferred to a test tube containing 2 ml of Conc. H_2SO_4. Formation of a reddish-brown color ring at the junction of two layers indicates the presence of glycosides.
b) **Bromine water test:** Each extract is added with bromine water, formation of yellow color precipitate indicates positive test for glycosides.

Tannins

a) **Gelatin test:** The concentrated methanolic extract of *C. montana* leaf was dissolved in water and mixed with 1% gelatin solution. Formation of white precipitate indicates the presence of tannins
b) **Ferric chloride test:** To 5 ml of the extract a few drops of ferric chloride was added. Development of black precipitate indicates the presence of tannins.

Steroids

a) **Salkowski test***:* Few ml of the extract $CHCl_3$ was added followed by the addition of Conc. H_2SO_4. Formation of red colour shows the positive test for steroidal compounds.
b) **Liebermann's Burchard test:** Few ml of the extract is dissolved in anhydrous $CHCl_3$ and treated with 0.5 ml of acetic anhydride followed by adding Conc. H_2SO_4 along the sides of the test tube. Formation of green colour indicates the presence of steroids.

Lignins

Lignin test: The plant extract was added with 2 ml of conc. HCl and 2 ml of 2% furfuraldehyde. Appearance of red color infers the presence of lignin.

Saponins

The aqueous extract was shaken vigorously and kept for 5 min without disturbing the solution. The persistence of honeycomb froth indicates the presence of saponins.

Anthocyanidins

The extract was added with methanolic HCl. The development of red or purple color indicates the presence of anthocyanidins.

Anthraquinones

The extract (3ml) was added with dilute H_2SO_4. Then the solution was boiled and filtered. The filtrate was cooled and an equal volume of benzene was added. The resultant solution was shaken well and the organic layer was separated which was added with equal volume of dilute Ammonia solution. Development of pink color indicates the presence of anthraquinones

Terpenoids

a) **Salkowski test**: Few ml of the extract was added with $CHCl_3$ followed by the addition of Conc. H_2SO4. Formation of lower yellow acid layer indicates the presence of Terpenoids
b) **Liebermann's Burchard test:** Few ml of the extract is dissolved in anhydrous $CHCl_3$ and treated with 0.5 ml of acetic anhydride followed by adding Conc. H_2SO_4 along the sides of the test tube. Formation of lower red color indicates the presence of Terpenoids.

Reducing sugars

Fehling‟s test: Filtrate is added with Dilute Hydrochloric Acid, Test for anthocyanidins neutralized with Alkali and heated with Fehling‟s A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.4 GC-MS analysis

5 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 recognised 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.

2.5 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 (Hagerman et al., 1998).

2.6 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 (Prieto *et al.* 1999).

2, 2- Diphenyl-1-picrylhydrazyl (DPPH) 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 (12) Yaushisakono, 1978)..

2.7 Antimicrobial Activity – Disc Diffusion Method (Bauer *et. al.* 1966)

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.

3. Results

Qualitative analysis of Phytochemicals: The preliminary screening of secondary metabolites showed the presence of 10 to 12 phytoconstituents in all the selected plant extracts as Alkaloids, steroids, lignins, flavonoids, phenols, terpenoids, tannins, cardiac glycosides, anthocynidins, reducing sugars and saponins.

Identification of phytoconstituents from the fractions through GC- MS studies:
Gas chromatography and mass spectroscopy analysis of methanolic stem bark of *C. divaricata*, has revealed the presence of bioactive phytoconstituents like terpenoids such as monoterpenes, sesquiterpenes, diterpenes, steroids and phenolic compounds. GCMS analysis of *C. divaricata* stem showed presence of 11 peaks (Fig.) corresponding to Pterin-6- carboxylic acid, 1,3-bis-t-butylperoxy phthalan, Phloroglucinol, 2,6-lutidine 3,5- dichloro-4- dodecylthio-, Propanedioicacid,propyl,. (2s,3s)-(-)-3-propnolyloxirane-methanol, Propanamide, 2-hydroxy, 1-adamantanemethylamine, α-methyl, Ethylamine,2-(adamantan- 1-yl)--methyl-, Ala-β-ala,trimethylsilyl ester, Urea, heptyl- (Table 1, Figre 2).

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

S. No	RT	Name of the compound	Biological Activity
1	6.180	Propanamide, 2-hydroxy	17-β-hydroxysteroid dehydrogenase-Inhibitor, Aryl-Hydrocarbon-Hydroxylase-Inhibitor,Testosterone- Hydroxylase-Inducer
. 2	26.828	Pterin-6-carboxylic acid	Acidifier, acidulant, inhibits production of uricacid, arachidonic acid Inhibitor
3	27.013	1,3-bis-t-butylperoxy phthalan	Antidote, antitumor(Thyroid), bloodthinning, Demulcent, Tonic, Testosterone-Inducer, TNF Inhibitor
4	27.138	2,6-lutidine 3,5-dichloro-4- dodecylthio	-
5	27.438	Propanedioic acid, propyl	Acidifier, acidulant, inhibits production of uric acid,arachidonic acid inhibitor
6	27.653	(2s,3s)-(-)-3-propnolyloxirane methanol	Analgesic-synergist, Antioxidant synergist stimulant, anticancer (skin,stomach), anti- diabetic antidote,
7	27.758	Phloroglucitol	Spasmolytic agent to treat colic, spasmtic pain of digestive and biliary tracts
8	27.964	1-adamantanemethyl amine, α.-methyl-	TNF-α-Inhibitor, α- Reductase-Inhibitor, Antimicrobial
9	28.119	Ethylamine,2-(adamantan-1-yl)-1-methyl-	No reports
10	28.229	Ala-.β.-ala, trimethylsilyl ester	17-β-hydroxysteroid dehydrogenase-Inhibitor, β-2- Receptor-Agonist, β-Adrenergic Receptor Blocker
11	28.774	Urea, heptyl-	Urealytic, Urease-inhibitor

#	RT	Scan	Height	Area	Area %	Norm %
1	26.828	4804	1,957,379	45,223.0	5.999	34.02
2	27.013	4841	1,692,312	96,821.1	12.843	72.84
3	27.138	4866	2,101,108	66,554.1	8.828	50.07
4	27.438	4926	2,022,201	50,102.8	6.646	37.69
5	27.653	4969	2,440,203	132,929.0	17.633	100.00
6	27.758	4990	1,220,864	48,983.7	6.498	36.85
7	27.964	5031	1,849,893	78,925.9	10.469	59.37
8	28.119	5062	1,696,049	66,398.1	8.807	49.95
9	28.229	5084	2,021,949	66,241.8	8.787	49.83
10	28.774	5193	1,894,969	101,702.3	13.490	76.51

Figure 2: GCMS analysis of Methanolic stem bark extract

Total Phenolic Content:
The aqueous extract showed high phenolic content of 224 mg/g dry wt. than the alcoholic extract (66 mg/g dry wt). (Table 3.).

Table 3: 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)
Aqueous extract	224±03	5.8±0.3	88±03
Alcoholic extract	66±02	4.8±0.2	111±05

Antioxidants:
Highest total antioxidants equivalents of ascorbic acid (vit.C) was observed in alcoholic extract (5.8mg/g dry weight) than aqueous extract (4.8 mg/g dry weight) and the (Table 3.)

Antioxidant activity

DPPH Scavenging Activity: *C. divaricata*, aqueous and alcoholic extracts of stem bark showed 50% scavenging at 88 µg and 111 µg respectively (Table 3.)

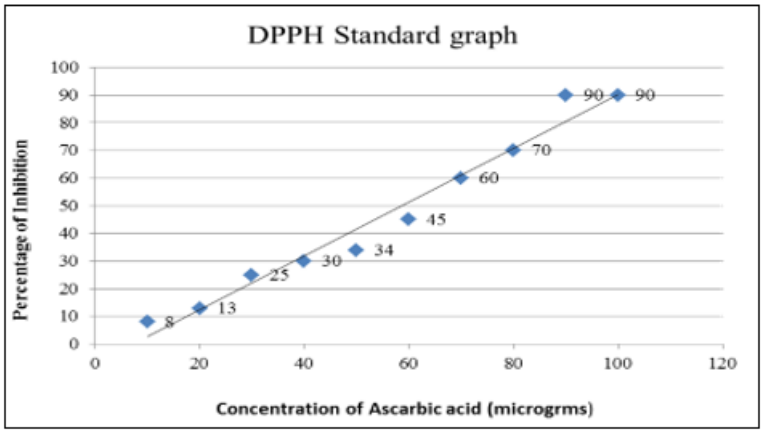

Figure 3: DPPH Standard Graph with Ascarbic acid (Vitamin C)

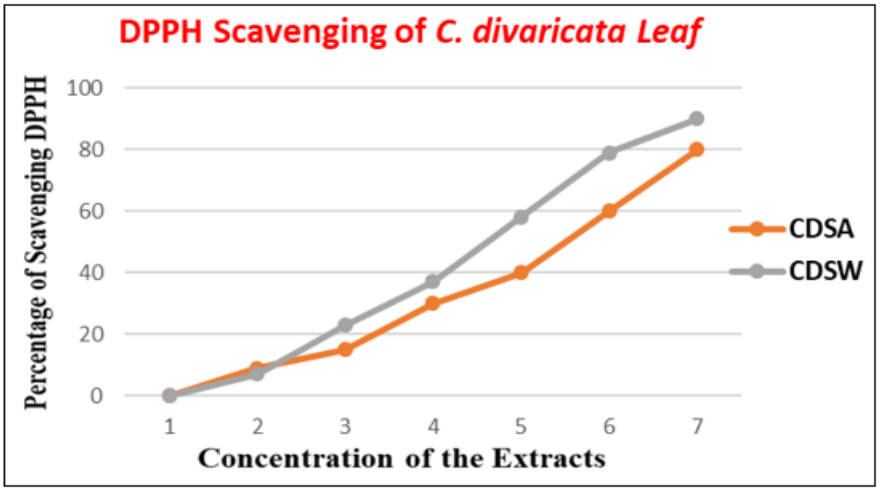

Figure 4: DPPH Standard Graph with Ascarbic acid (Vitamin C)

Antibacterial Activity
Aqueous leaf extract showed antimicrobial activity on *S. dysenteriae.* No activity was observed on *B. subtilis, K. pneumoniae and P. aeruginosa* (Table.4)

Table 4: Antibacterial activity *C. divaricate* Stem Bark (Zone of Inhibition in mm)

S. No	Microorganism	Ethanol (µg/disc)				Aqueous (µg/disc)				Standard Antibiotic*
		25	50	75	100	25	50	75	100	100
1	*B. subtilis*	-	-	-	-	-	-	-	-	22
2	*K. pneumoniae*	-	-	-	-	-	-	-	-	16
3	*P. aeruginosa*	-	-	-	-	-	-	-	T	22
4	*S. decenteriae*	-	-	T	6.± 0.3	-	T	6.±0.5	8.4±0.5	20

Ab *Amaxillin, T: Trace

4. 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. The biological activities, phytochemical screening and antioxidant properties were discussed in *C. divaricate* stem bark. Bioactivity of *C. divaricata* stem bark Phytoconstituents: Propanamide, 2-hydroxy is used in the topical application of diseases such as dry skin, eczema, and plantar hyperkeratosis, dandruff, acne and warts (Ruey and Van Scott 1995). Antimicrobial and antiviral screening of novel indole carboxamide and propanamide derivatives revealed Propanamide derivatives studied for antimicrobial and antiviral efficacy (Ölgen et al. 2008). The study of chemical composition of *Foeniculum vulgare* revealed that Pterin 6 carboxylic acid Anti-cancer, anti-viral, Antipsychotic, mood stabilizer and anti-parasite, antipsychotic (Hussein *et al*,2016). Phytochemical, in vitro and in silico screening of roots of *Jasminum auriculatum* for antioxidant activity revealed the presence of 1,3-bis-t-butylperoxy-phthalan which was reported to bioactivities like Anti-tumor, catechol o methyltransferase, Glutathione transferase inhibitor, Tachycardiac (Sridevi et al 2022). GCMS analysis of chemical constituents and in vitro antioxidant activity of the organic extracts from the stem of *Bridelia stipularis* was reported that (2S, 3S)-(-)-3-propyloxirane methanol was predicted to have antioxidant activity (Yusufzai et al. 2019). (2s,3s)- (-)-3-propyloxirane methanol reported to possess biological activities such as Analgesic stimulant, anticancer (skin, stomach), anti-diabetic antidote (Dr. Duke's Database, 2015). Research on the role of Phloroglucinol in irritable bowel syndrome proved its efficacy as an antispasmodic agent (Jafri *et. al.* 2006). Research on the relationship between phloroglucinol and oxidative stress showed that phloroglucinol inhibited the production of inflammatory mediators in RAW264.7 cells including prostaglandin E2, interleukin-1β, interleukin-6 and tumor necrosis factor-α. Also phloroglucinol reduces the expression of matrix metalloproteinase, which is crucial for chronic inflammation, in HT1080 cells, a human fibrosarcoma cell line. (Kim and Kim 2010), A study on Phloroglucinol attenuates DNA Damage and Apoptosis induced by Oxidative Stress in Human Retinal Pigment Epithelium ARPE-19 Cells by Blocking the Production of Mitochondrial ROS, showed the therapeutic potential of phloroglucinol to prevent oxidative stress-mediated damage in RPE cells. Phloroglucinol attenuates motor functional deficits in an animal model of Parkinson's disease by enhancing Nrf2 activity (Ryu *et al.* 2013). Novel anticancer activity of phloroglucinol against breast cancer stem-like cells revealed that Phloroglucinol can be used to target breast cancer stem-like cells and to prevent the disease relapse (Kim *et al.* 2014). A controlled double-blind trial using household setting revealed that α-methyl-1-adamantane methylamine decreased the severity of influenza A2 infections in humans (Donald 1970). antibacterial which can be used in the manufacture of various kinds of levofloxacin tablets (Hayden 1992), a strong inhibitor of *Sindbis vir us* reproduction (26 Raval 2015), Ala-β-ala, trimethylsilyl ester, acts as anti-amyloid, androgenic. Urea, heptyl- is a ureolytic and urease inhibitor (Dr. Dukes database 2015).

5. Conclusion

The present investigation on the phytochemical evaluation and biological activities revealed that, the extracts were effective against the ethnobotanical claims made by the local tribes. Further in vivo evaluation required to confirm the assumed medicinal properties.

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