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Research Article **Antibacterial Studies**

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PHYSICOCHEMICAL, PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL INVESTIGATION ON LEAVES OF *CARDIOSPERMUM CANESCENS* WALL.

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1. ABSTRACT

Effect of ethanol, methanol and petroleum ether extracts from the leaves of *Cardiospermum canescens* Wall. was studied against human pathogenic bacterial strains of *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia* and the antibiotic sensitivity of bacterial strains was also analyzed by using Bauer–Kirby method. Finally leaf powder was subjected to physicochemical and preliminary phytochemical evaluations to establish the quality and purity of the drug. In the Physicochemical studies, loss on drying, total ash value, acid insoluble ash, water insoluble ash, various extractive values etc., were carried out. Further, qualitative tests for various functional groups like triterpenoids, alkaloids, glycosides etc., were carried out. HPTLC profile was also established with ethyl acetate, and methanolic extracts. The results obtained from the study concluded that leaf powder of this plant is a great potential source as an antibacterial agent and preliminary phytochemical studies revealed the presence of triterpenoids, alkaloids, tannins, flavonoids and proteins.

2. KEY WORDS: Medicinal Plants, Antibacterial activity, Ethanolic and Methanolic and Petroleum Ether Extracts

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3. INTRODUCTION

India is a big tropical country and a home of an extraordinary variety of climatic regions, ranging from tropical in the South to temperate and alpine in the Himalayan North, where elevated regions receive sustained winter snowfall. The India's climate is strongly influenced by the Himalayas and the Thar Desert.(Joy, et al. 2001 and Chatterjee and Bandhaba 1953). By having these climatic regions India becomes a treasure of rich Biological diversity in plants, animals and microorgani -sms. Since the beginning of human life in Indian sub continent man has been facing several diseases by different microorganisms. To get relief from these remedies, ancient people disposed several plant based drugs with trial and error basis. With this knowledge traditional systems like Ayurveda, Siddha and Ethnomedicine acquired their birth in India. Now this knowledge is bounded in tribal hamlets only due to urbanization and employing the synthetic drugs in large scale. In Comparison with herbal drugs, synthetic drugs are hazardous and cause side effects. To reveal this knowledge, author did an ethnobotanical survey from 25th June to 25th July 2011 in Nellore district of Andhra Prad-esh, India. This area is mainly inhabited by three types of tribal groups, these are Yanadi, Chenchu and Nakkalas(Penchala et al. 2010). They have been living in thick forest zones from immemorial days having no other source of getting modern medicine except herbal medicine for their health care. By their habitat they are commonly met with severe fevers. For getting relief from these fevers they have been widely using the medicinal plant *Cardiospermum canescens* Wall. (Leaves). With this view it is put to systematic scientific investigation in the present study. This species is globally distributed in the Pantropics. Within India, it is found in the tropical and subtropical regions throughout the plains, ascending upto an altitude of 1300 m. (Penchala et al. 2012).

3.1 Taxonomy of the plant

Climbing tendril- bearing herb, up to 5m, with wiry stem and branches, stems deeply furrowed. Leaves biternate; leaflets ovate-lanceolate, 1.5-5x 1-3 Cm. pubescent, base acute or attenuate apex acuminate, petiolule to 1 mm. Flowers white, in long peduncled umbellate cymes on tendrils. Male flowers: sepals suborbicular, 1.5 mm. petals 4, white, 6X4 mm; Capsules bloated not winged, 3.5 X 3 Cm; seeds globose, to 5mm (Fig. 3)(Gam-ble, 1967; Kirtikar and Basu. 1999)

4. MATERIALS AND METHODS

4.1Plant collection and processing

Fresh leaves of ***Cardiospermum canescens* Wall.** were collected from the tribes of Nellore district. Identification and confirmation were done by Department of Botany Sri Ven-kateswara University. The voucher herbarium specimen was also deposited in the Department of Botany Sri Venkateswara Uni-versity(Jain, 1977),(Penchala et al. 2012). The leaves were washed with running tap water and air dried under shade for five days. After five days the leaves were macerated with mixer grinder to yield fine powder. This dried powder, about 50 grams, was extracted with soxhlet apparatus using 100 ml of absolute Methanol, Ethanol, Chloroform, water, Benzene, and Petroleum ether separately**.** These obtained extracts were stored at 4^{0}C.(Archana et al. 2010)

4.2 Bacterial cultures and control

Three bacterial species, *Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia* were obtained from Microbiology department of Sri Venkateswara University, Titupati, Chitt-oor district of Andhra Pradesh, India.

4.3 Inoculum preparation

Bacterial inoculums were prepared with above mentioned three bacterial species by main-taining the temperature with 4^{0}C on nutrient agar slants. These agar slants were prepared by inoculating a loop full of bacterial culture into a 4 ml. sterile nutrient bath and incubated for 24 hours at 35^{0}C. After incubation a little amount of

bacterial culture was transferred to liquid nutrient media and incubated for 24 hours to increase cell density.

4.4 Antibacterial activity

The antibacterial activities of the leaves were tested against the selected bacterial strains by Bauer–Kirby Disc diffusion method. The 20 ml of sterilized agar medium was poured into sterile petriplates and allowed to solidify. The testing bacterial cultures from liquid media were spread over the solidified media by using sterile swab. 6-mm filter paper disks were prepared and impregnated on solidified media. ethanol, methanol and petroleum ether extra-cts at the concentrations of 20, 40, 60, 80 and 100% were poured on filter paper disks with micropipette. After pouring these plates were incubated at 37^{o}C for 24-48 hours. After the incubation period, the results were observed and the diameter of inhibition zone around the each paper disk was measured.

4.5 Composition of nutrient Agar medium

Chemicals Composition

1. Agar – 20 grams
2. Beef extract – 3 grams
3. Distilled water – 1000 ml
4. Peptone – 5 grams
5. Sodium chloride – 5 grams

5. RESULTS AND DISCUSSION

In the present study Methanolic, Ethanolic, Chloroform, water, Benzene, and Petroleum ether extracts from the leaves of *Cardiosper-mum canescens* Wall. were subjected for anti - bacterial investigations against three bacterial species. The three extracts methanolic, ethan-olic and petroleum ether showed the activity whereas other extracts chloroform, water, benzene showed negligible activity. The anti bacterial activity was also comparatively analyzed for sensitivity tests.

5.1 Antibacterial activity of Cardiospermum canescens Wall.

Ethanolic, methanolic petroleum ether extracts of *Cardiospermum canescens* Wall. showed maximum zone of inhibition against *Klebsiella pneumonia* 15 mm, 25 mm and 28 mm; *Salmonella typhi* 15 mm, 24 mm and 29 mm; *Escherichia coli* 17 mm, 24 mm and 26 mm in diameter. (Table1,2&3). With this data it is confirmed that ethanolic leaf extracts have higher antibacterial activity when compared with methanolic and petroleum ether extracts. (Fig. 4,5&6)

5.2 Antibiotic sensitivity test

Antibiotic discs were obtained commercially from Indian scientific company, Tirupati, Andhra Pradesh. All antibiotics were taken at the concentration of 30µg.The abbreviations of antibiotics were given in brackets as Ampicillin(A);Amoxicillin(Am);Chloramphenicol(C);Erythromycin(E);Penicillin(P);Kanamycin(K); Tetracycline(T);Cephalexin(Cp);Norfloxacin(Nx); Ciprofloxacin(Cf.).The antibiotic sensitivity of three bacterial species was studied by using Bauer–Kirby Disc Diffusion method. A sterile swab was dipped into liquid media which was already incubated with bacteria and streaked three times on the surface of solidi-fied agar in petriplate. After this five Antibiotic disc were placed and incubated for 24 hours at 37^{0}C. after incubation, plates were studied for zone of inhibition. The results of antibiotic sensitivity test are presented in Table 4. The results revealed that all the antibiotics exhi-bited higher antibacterial activity and compa-ratively the leaf extracts of *Cardiospermum canescens* Wall. were also exhibited higher antibacterial activity against *Salmonella typhi.*

5.3 Leaf powder was subjected to *physico-preliminary phyto chemical evaluations to establish the quality and purity of drug*

5.4 Fluorescence study:

Fluorescence analysis were studied and pres-ented in Table 5. The fluorescence characters of powdered drug play a vital role in the determination of quality and purity of the drug material. In the present study, powder treated with various reagents showed characteristic fluorescence at 255 nm and 365 nm wavele-ngth (Penchala et al. 2012).

5.5 Physico-chemical details (Kokoski et al, 1958 and Chase et al, 1949)

To determine extent of adulteration as well as to establish the quality and purity of drug, ash values were calculated (Penchala et al. 20-12).Total ash was found to be 9.5% of which 1.12% was acid insoluble ash, 2.25% was water soluble ash and 5.9% water insoluble ash. The extractive values were found to be 21.52% and 16.43% for water and alcohol respectively, which indicated higher extractive value for water compared to alcohol. The moisture content was found to be 21.64%. The results are given in Table 6.

5.6 Preliminary phytochemical tests *(Indian Pharmacopoeia 2007)*

These tests revealed the presence of Triterpenoids, Flavonoids, Alkaloids, Saponins, Proteins, Carbohydrates, and Tannins listed in Table 7.

5.7 HPTLC analysis

A densitometric HPTLC fingerprint was established with ethyl acetate and methanolic extract for the development of characteristic fingerprint profile, which may be worked as marker for quality evaluation and standardization of the drug. Rf values and the relative percentage of the separated compounds are given in Table 8.HPTLC fingerprint and densitogram are given in Fig. 1 and Fig. 2 respectively.

Table 1

Effect of Methanolic Extracts of Leaves on control of Bacterial species

		100 µl of Methanolic extracts(conc.mg/ml)				
S.No	**Bacterial species**	20%	40%	60%	80%	100%
1.	*Escherichia coli*	08mm	10mm	13mm	15mm	17mm
2.	*Salmonella typhi*	07mm	08mm	11mm	13mm	15mm
3.	*Klebsiella pneumonia*	09mm	09mm	10mm	14mm	15mm

Table 2

Effect of Petroleum Ether Extracts of Leaves on control of Bacterial species

		100 µl of Petroleum ether extracts(conc.mg/ml)				
S.No	**Bacterial species**	20%	40%	60%	80%	100%
1.	*Escherichia coli*	13mm	15mm	20mm	22mm	24mm
2.	*Salmonella typhi*	12mm	17mm	21mm	23mm	24mm
3.	*Klebsiella pneumonia*	14mm	18mm	21mm	22mm	25mm

Table 3

Effect of Ethanolic Extracts of Leaves on control of Bacterial species

		100 µl of Ethanolic extracts(conc.mg/ml)				
S.No	**Bacterial species**	20%	40%	60%	80%	100%
1.	*Escherichia coli*	14mm	17mm	19mm	22mm	26mm
2.	*Salmonella typhi*	13mm	16mm	22mm	26mm	29mm
3.	*Klebsiella pneumonia*	15mm	17mm	21mm	24mm	28mm

Table 4
Antibiotic Sensitivity of Bacterial species (Zone of inhibition in mm diameter)

S.No	*Bacterial species*	A	Am	C	E	P	K	T	Cp	Nx	Cf
1.	*Escherichia coli*	18	22	23	19	16	19	21	16	34	32
2.	*Salmonella typhi*	19	21	21	18	19	20	20	13	22	23
3.	*Klebsiella pneumonia*	20	22	24	20	21	22	19	19	30	30

Table 5
Behaviour of the Drug (Leaf) Powder with different Chemical Reagents (Kokoski et al,1958 and Chase et al,1949)

S.N0.	**Treatment**	**Colour observations Under**		
		Ordinary light	**U.V Light**	
			255nm	**365nm**
1.	Powder +Distilled water	Light Green, mucilaginous with foamy nature.	Dark Green	Green
2.	Powder+5%Aqueous $FeCl_3$	No change	No change	No change
3.	Powder+Glacial acetic acid	No change	No change	No change
4.	Powder+5% HNO_3	No change	No change	No change
5.	Powder+N/10 Iodine Solution	Blue colour	Dark Blue	Pale Blue
6.	Powder+ConHCl	Light Green	Dark Green	Green
7.	Powder+ConH_2SO_4	Black	Black	Brownish Black
8.	Powder+Ammonia solution	Light green	Dark Green	Green
9.	Powder+5%Aqueous NaOH	No change	No change	No change
10.	Powder+5%Aqueous KOH Solution.	Green	Black	Blackish green

Table 6
Physicochemical Parameters (% W/W)

S.No	**Reaction Cardiospermum canescens:**	**Values**
1.	**Total ash**	9.5%
2.	**Acid insoluble ash**	1.12%
3.	**Water soluble ash**	2.25%
4.	**Water insoluble ash**	5.9%
5.	**Moisture content (LOD) at 110 C**	21.64%
6.	**Water soluble extractive values**	21.52%
7.	**Alcohol soluble extractive values**	16.43%

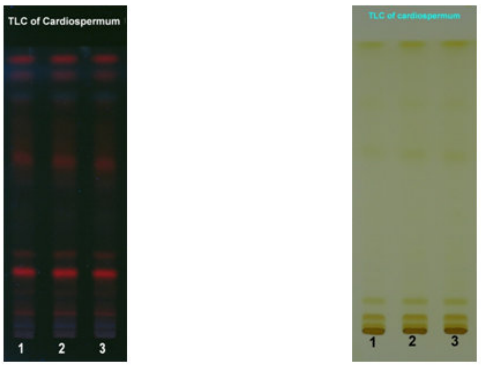
Table 7
Preliminary Phytochemical Tests revealed the Presence of Triterpenoids, Flavonoids , Alkaloids , Proteins and Tannins

S.No	**Chemical compound** *Cardiospermum canescens*	**Chemical test**	**Result**
1.	**Triterpenoids**	a)Leibermann buchard test	+
		b)salkowsky test	+
2.	**Flavonoids**	a)Lead acetate test	+
3.	**Glycosides**	a)Baljet test	_
		b)Legal test	_
4.	**Steroids**	a) Leibermann buchard test	_
		b)Salkowsky test	_
5	**Alkaloids**	a)Dragendroffs test	+
		b)Hagers test	+
		c)wagers test	-
		d)Mayers test	+
8.	**Proteins**	Biuret test	+
9.	**Tannins**	Ferric chloride test	+

Table 8
Peak List of *Cardiospermum canescens* Wall. at 366nm

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1	10.7	1493.80	67.9	738.09	0.02
2	20.7	5.58	0.3	4.76	0.16
3	25.8	123.37	5.6	53.57	0.23
4	30.3	13.67	0.6	6.92	0.30
5	40.2	62.75	2.9	21.91	0.43
6	47.5	3.74	0.2	2.54	0.53
7	53.8	80.92	3.7	22.41	0.62
8	67.8	13.12	0.6	6.15	0.82
9	78.2	402.55	18.3	81.62	0.96

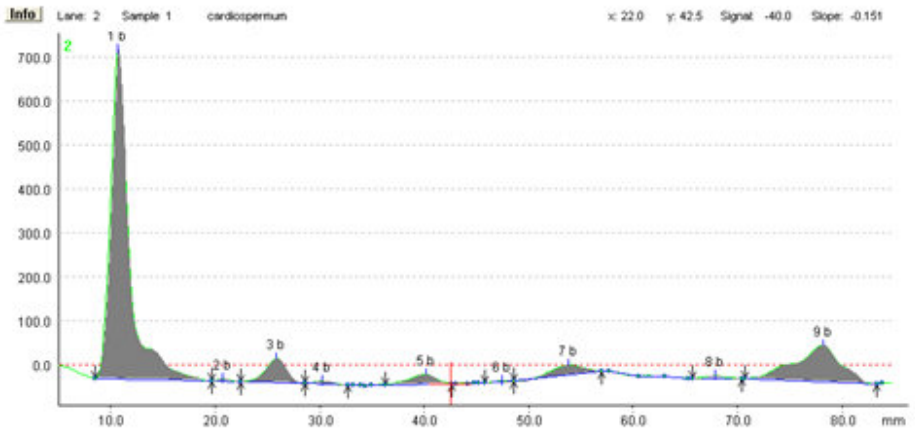
Figure 1
Finger Print TLC of Methanolic extract of Cardiospermum canescens* Wall. *applied in triplicate as Track 1, 2 & 3(Krebs,1969)



UV 366nm ***Under Iodine vapors***

Solvent system: Toluene: Ethyl Acetate: Methanol = 7: 2: 1 Spots: Nine under UV 366nm Solvent Run: 81mm

Figure 2
Densitogram of *Cardiospermum canescens* Wall. at 366nm



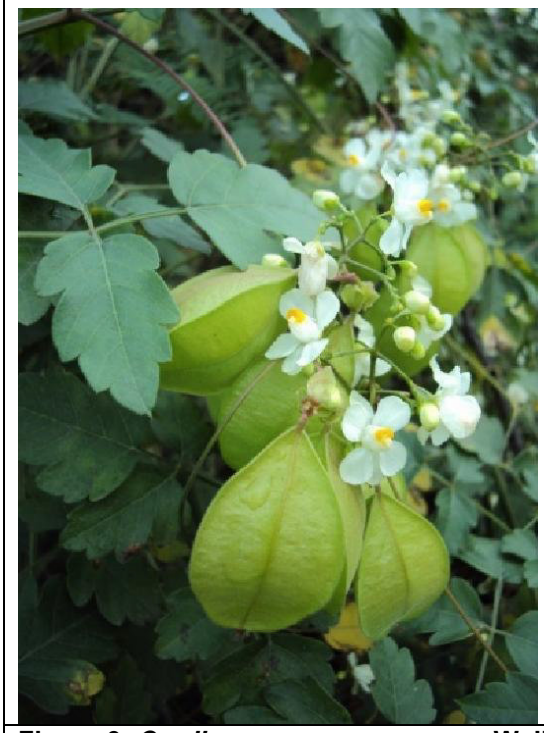


Figure 3: ***Cardiospermum canescens*** **Wall.**

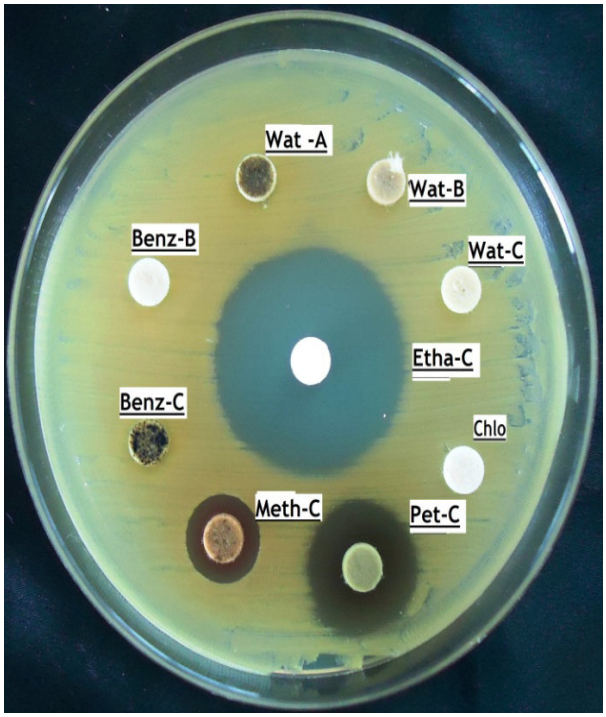


Figure 4: ***Inhibition Zones by different extracts with Salmonella typhi***

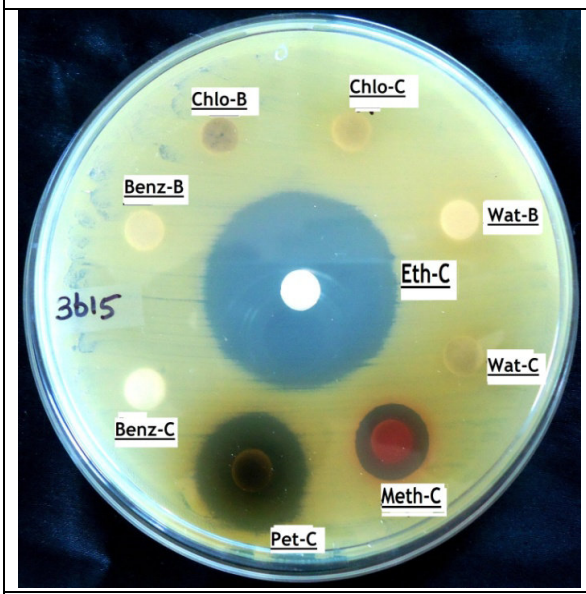


Figure 5: ***Inhibition Zones by different extracts with Klebsiella pneumonia***

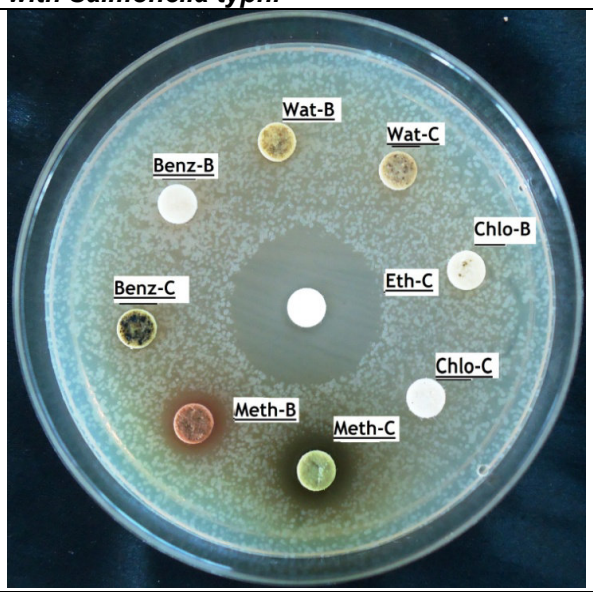


Figure 6: ***Inhibition Zones by different extracts with Escherichia coli***

Wat: Water extract; Chlo: Chloroform extract; Pet: Petroleum ether extract; Benz: Benzene extract; Etha: Ethanolic extract and Meth: Methanolic extract. All the extracts were taken 100 µl. A: 25%, B: 50% and C: 100% concentrations.

6. CONCLUSION

From the results, antibacterial screening of ethanolic and methanolic extracts of leaves, clears that the drug(Leaves) is a potential source for antibacterial studies and physicochemical and preliminary phytochemical evaluations assist as an important source of information to ensure the identity and to determine the quality and purity of the plant material in future studies.

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