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REVEALING THE MEDICINAL VALUE OF CUSCUTA REFLEXA THROUGH ITS BIOCHEMICAL PROFILING

AMNA ILYAS[1], AMINA ARIF[1], AIHSA SETHI[3], SHAFIA ARSHAD[2*], UZMA BASHIR[2], RIDA SIDDIQUE[3], MARYAM FURRAKH[3], USMAN WAJID[1]

[1]Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab.
[2]University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Pakistan
[3]Faculty of Pharmaceutical Sciences, Government College University Faisalabad, Pakistan
*Corresponding author Email: shafia.arshad@iub.edu.pk

Abstract

The parasitic herb Cuscuta reflexa is perennial, member of the “Convolvulaceae” family, golden yellow and leafless. Cuscuta reflexa, called dodder, is a holoparasite with a very low level of chlorophyll and photosynthesis activity. Qualitative and quantitative analysis on the methanol extract and its fractions were performed. Total phenolics content using Folin-Ciocalteu reagent and total flavonoid content by aluminium chloride assay was measured. Methanol extract was analyzed through gas chromatography-mass spectrometry. Biological activities including anti-oxidant, anti-diabetic, anti-inflammatory, anti-bacterial, hemolytic, and thrombolytic activities carried out at different concentrations of these extracts to measure the potential of each concentration. Qualitative analysis confirmed the presence of many biological compounds i.e. tannins, terpenoids, steroids, phenols, glycosides. Ethyl acetate fraction found to be had highest total phenolic (34 ± 0.026) and total flavonoid content (87 ± 0.014) value. Gas chromatography-mass spectrometry confirmed the presence of many bioactive chemicals. Cuscuta reflexa has shown to have significant biological properties. Methanol extract and its ethyl acetate fraction showed significant zones of inhibition against gram positive (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae). Staphylococcus aureus was the most sensitive one than other bacteria used to test. It is concluded in this study that Cuscuta reflexa can be effectively used to develop novel drugs against bacterial infections, inflammation, diabetes, and oxidation.

Key words: Cuscuta Reflexa, oxidation, Phytochemicals, Biological activities, Extraction

1. INTRODUCTION

Plants and plant products play a significant role as sources of medicine. The majority of modern medications are derived from plants (Süntar, 2020). Our understanding of the active ingredient and medicinal components in plants is constantly being updated through various biochemical techniques. Numerous techniques, including Thin-Lay Chromatography, HPTLC, HPLC, NMR, FTIR and XRD, are utilized to examine plants' different biochemical and chemical aspects (Kotha & Luthria, 2019). To determine the bioactive components of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, etc., the optimum method is GC-MS. GC-MS is the best method for qualitative investigation of volatile and semi-volatile bioactive chemicals because it combines the best separation (GC) and identification (MS) techniques (Majchrzak et al., 2018). Many medicinal plant parts are used to make extracts, each having unique therapeutic properties. In order to do this, secondary metabolites produced

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by plants had to be isolated, identified, and used as active ingredients in pharmaceutical formulations (Seca & Pinto, 2018). The scientific communities studying biology have recently been interested in traditionally utilized therapeutic herbs. In the current study, Cuscuta Reflexa extracts in methanol was subjected to GC MS analysis and different polarity solvent fractions form were screened for phytochemicals and biological activities.

2. METHODOLOGY

2.1 Collection of plant

The parasitic plant Cuscuta reflexa was collected in March from Lahore, Pakistan. Parasitic plant was identified and confirmed from a Botanist. All experiments were performed in Biochemistry lab of University of Central Punjab, Lahore.

2.2 Extraction and Fractionation

Collected plant was washed to remove all the dirt and extraneous matter, then it was shade dried completely for 6 to 8 days. Dried plant was then ground into fine powder by using mechanical grinder. 200 g of powder was used for extraction by using maceration process, powder was transferred into conical flask which contained 1 L methanol. Flask was left for 10 days in shaking incubator, then it was followed by filtering the contents of flask first through Muslin cloth and then filter paper. Collected filtrate was then dried, it was evaporated at rotary evaporator at 40° C. Obtained extract color and yield was noted. Methanolic crude extract was used to make fractions using solvents n-hexane, ethyl acetate, and chloroform.

2.3 Phytochemical Analysis

Analysis for identification of the major phyto-constituents was carried out as follows:

2.3.1 Qualitative analysis: Various phytochemicals of methanol extract of C. Reflexa and its three fractions of different polarity solvents (n-hexane, ethyl acetate, chloroform) including flavonoids, saponins, terpenoids, phenols, glycoside, steroids and tannins was detected in plant extract preparation by standard methods (Munir et al., 2022).

2.3.2 Quantitative estimation of phytochemicals

2.3.2.1 Total phenolic content (TPC): The Folin-Ciocaletu reagent method was performed to find out the TPC in plant extracts (Jain et al., 2014).

2.3.2.2 Total flavonoid content (TFC): Total flavonoid content was determined by following the method (Bilal, Parveen, Fiaz, & Mazhar, 2022).

2.4.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

For the GC, an Agilent USA instrument was employed. 50 °C starting column temperature and 1-minute hold duration were used. The temperature was set to increase by 8°C every minute, reaching a maximum of 280°C. One microlitre of the sample was injected into the port, where it instantly vaporized and transported down the column at a flow rate of one millilitre per minute using helium as the carrier gas. At 70 eV, the MS spectrum was recorded. Following the column separation, the components were identified and

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subjected to further analysis using flame-ionization detection (FID). By comparing the spectra of unknown chemicals with the spectrum of known compounds in PubChem, the compounds' molecular weights, structures and names were determined.

2.5 Biological Activities of C. Reflexa

2.5.1 DPPH radical scavenging activity

For the evaluation of free radical scavenging activity of the extract 2.2 diphenyl-1-picrylhydrazyl (DPPH) method was used as described by (Bilal et al., 2022) with some alterations. Following formula was used to calculate the radical scavenging activity.

$$\textbf{Percentage radical scavenging activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.5.2 Anti-diabetic activity

Starch iodine method was used to measure the activity of alpha-amylase inhibition as described by (Ononamadu et al., 2020) with modifications.

Following formula was used to calculate the percentage of inhibition.

$$\textbf{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5.3 Anti-inflammatory activity

Anti-inflammatory activity of the methanol extract of C. Reflexa and its three fractions (n-hexane, ethyl acetate, chloroform) was assessed using the method described by (Naz et al., 2017) with modifications. Values taken in triplicates.

Following formula was used to calculate the percentage of inhibition.

$$\textbf{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{x}100$$

2.5.4 Hemolytic activity

Hemolytic activity of C. Reflexa was assessed by using method described by (Kumar, Karthik, & Rao, 2011) with slight modifications. Following formula was used to measure hemolytic assay.

$$\textbf{Percentage hemolysis} = \frac{\text{Absorbance of Sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control}} \text{x}100$$

2.5.5 Thrombolytic Activity

Thrombolytic activity of C. Reflexa was assessed by using the method described by (Hussain, Islam, Bulbul, Moghal, & Hossain, 2014) with modifications.

Weight of clot = (weight of tube containing clot – weight of tube alone)

% Thrombolysis = (weight of clot before lysis – weight of clot after lysis) x 100

2.5.6 Antibacterial activity

Antibacterial assay of C. Reflexa samples was performed. Both gram negative and gram positive strains were used to find out the antimicrobial activity by using well diffusion

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method. Staphylococcus aureus strain of bacteria was gram positive and Eschericia coli and Klebsiella Pneumoniae strain were gram negative according to (Deepthi, Renjith, Shameem, Habeeb Rahman, & Chandramohanakumar, 2022).

3. RESULTS

3.1 Screening of Phytochemicals

3.1.1 Qualitative Analysis of C. reflexa

Phytochemical analysis showed that phenols and tannins were present in methanol extract and its ethyl acetate fraction. Steroids and glycosides both were absent in n-hexane fraction. Terpenoids were absent in chloroform fraction.

Table 3.1: Phytochemical screening results of C. Reflexa by qualitative screening

Phytochemical Screening of Cuscuta reflexa					
Sr. #	**Tests**	**Plant Extracts**			
		Methanol	**n-hexane**	**Ethyl Acetate**	**Chloroform**
1.	**Flavonoids**	+	-	+	+
2.	**Steroids**	+	-	+	+
3.	**Phenols**	+	-	+	-
4.	**Tannins**	+	-	+	-
5.	**Saponins**	-	-	-	-
6.	**Terpenoids**	+	+	+	-
7.	**Glycosides**	+	-	+	+

(Presence of phytochemical is indicated by '+' whereas the absence is indicated by '-')

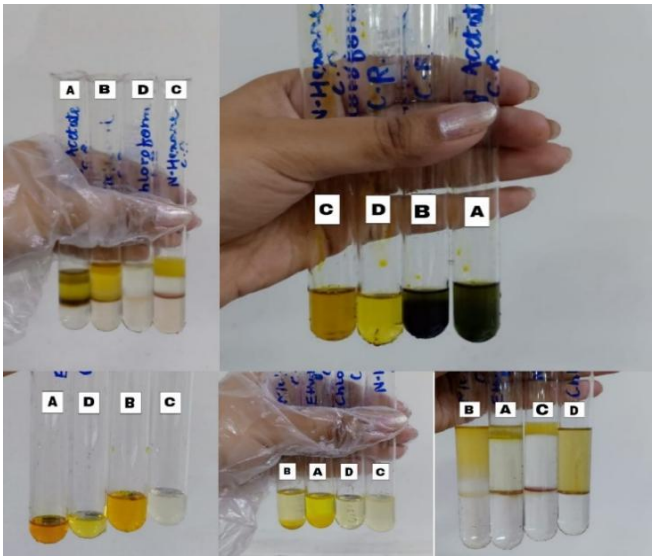

Figure 3.1: Qualitative tests of C. Reflexa extracts

A= Ethyl acetate, B= Methanol, C= n-hexane, D= Chloroform

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3.1.2 Quantitative Analysis of C. Reflexa

3.1.2.1 Total Phenolic Content

The numerical values attained after investigational effort for the total phenolic compounds along with different fractions were found to be higher in ethyl acetate fraction i.e. 34.08 ± 0.026 followed by n-hexane 32.81 ± 0.018, methanol 24.42 ± 0.017, and chloroform 23.50 ± 0.017 mg GAE/g.

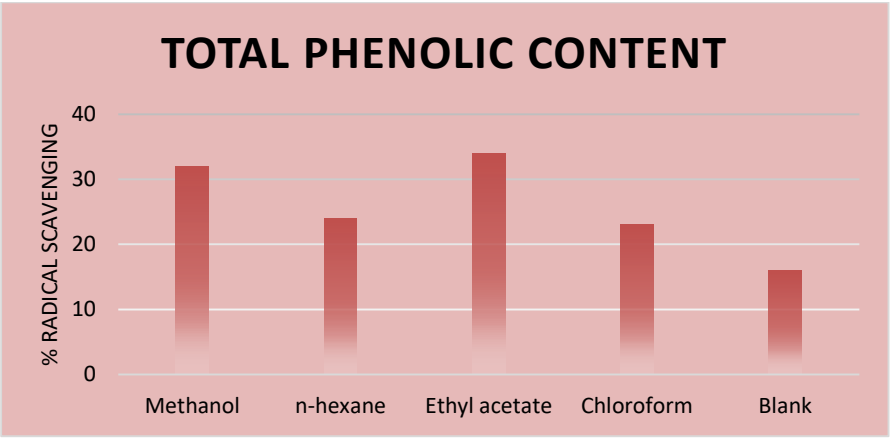

Figure 3.2: Amount of phenolic content in different fractions of C. Reflexa

In the above figure x-axis indicate the different fractions of C. Reflexa and y-axis showed the phenolic content of the plant.

3.1.2.2 Total Flavonoid Content

The numerical values attained after investigational effort for the total flavonoid compounds along with different fractions were shown higher in ethyl acetate fraction i.e, 34.08 ± 0.026 followed by n-hexane 32.81 ± 0.018, Methanol 24.42 ± 0.017, and Chloroform 23.50 ± 0.017 mg QE/g.

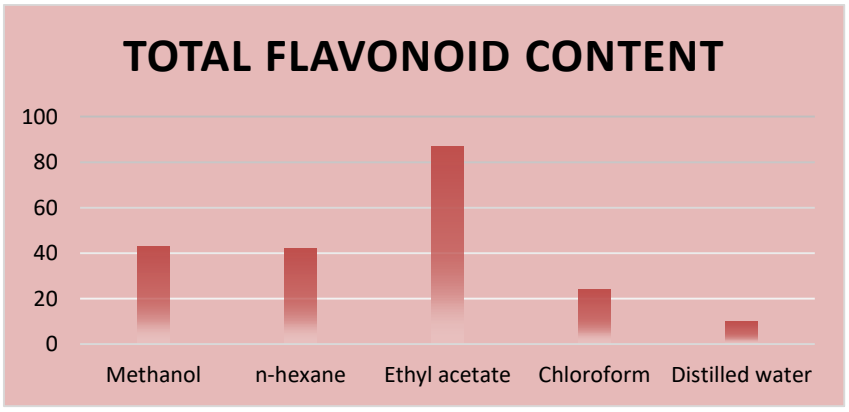

Figure 3.3: Amount of flavonoid content in different fractions of C. Reflexa

In the above figure x-axis indicate the different fractions of Cuscuta reflexa and y-axis showed the flavonoid content of the plant.

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4.2.3 Gas-Chromatography Mass-Spectrometry Analysis

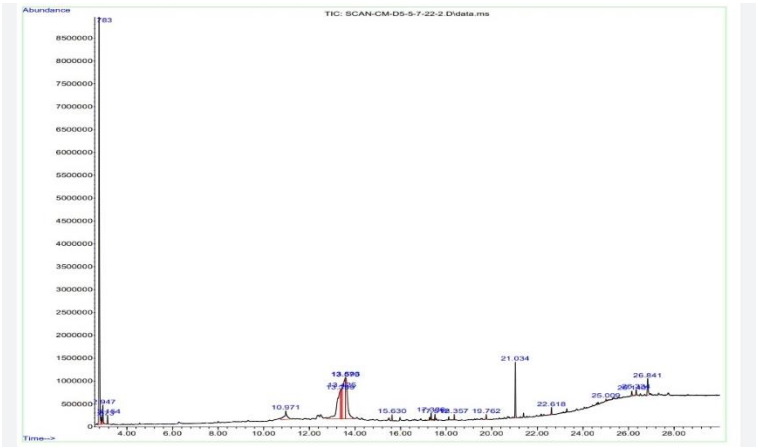

Figure 3.4: GC-MS chromatogram of methanol extract of C. reflexa

Table 3.2: Compounds identified from methanol extract of C. Reflexa

Sr. No	Names of Compounds	Molecular formula	Molecular weight (g/mol)	Retention Time (min)	Peak area (%)
1.	Chlorobenzene	C_6H_5Cl	112.56	2.783	37.14
2.	Ethylbenzene	C_8H_{10}	106.167	2.873	0.78
3.	1,3-dimethyl-benzene	C_8H_{10}	106.16	2.947	1.89
4.	p-xylene	C_8H_{10}	106.16	3.154	0.72
5.	Butylated hydroxytoluene	$C_{15}H_{24}O$	220.35	10.971	3.21
6.	3-O-Methyl-D-fructose	$C_7H_{14}O_6$	194.18	13.359	14.07
7.	Methyl 2-O-methyl-alpha-D-Xylofuranoside	$C_6H_{12}O_5$	164.16	13.425	4.71
8.	2-[2-[2-[2-[2-[2-[2-[2-[2-(2-Acetyloxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl acetate	$C_8H_{16}O_5$	192.21	13.575	14.72
9.	2-Trimethylsilyl-1,3-dithiane	$C_7H_{16}S_2Si$	192.42	13.593	12.38
10.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	15.630	0.44
11.	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264.4	17.336	0.64
12.	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-	$C_{30}H_{50}O$	426	17.512	0.42
13.	trans-Geranylgeraniol	$C_{20}H_{34}O$	290.5	18.357	0.43
14.	Farnesol formate	$C_{16}H_{26}O_2$	250.37	19.762	0.46
15.	Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	21.034	4.02
16.	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.556	22.618	0.61
17.	4-(4-Hydroxy-2,5-dimethylbenzyl)morpholine, TMS	$C_9H_{10}O_4$	182.17	25.009	0.19
18.	4-(7-Methyloctyl)phenol, TMS derivative	$C_{15}H_{24}O$	220.356	26.140	0.61
19.	Tetrasiloxane, decamethyl	$C_{10}H_{30}O_3Si_4$	310.68	26.334	0.61
20.	2-(Pyridin-2-ylformamido)acetic acid, 2TMS	$C_8H_8N_2O_3$	180.163	26.841	1.95

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3.2 Biological Activities of C. reflexa

3.2.1 Antioxidant activity

Table 3.3: DPPH Radical Scavenging activity of C. Reflexa

Sr. no	Sample	Concentration (mg/ml)	Mean ± S.D (DPPH inhibition percentage)	IC_{50} mg/ml
1	Methanol	3	69.2 ± 0.096	2.4096
		2.5	64.7 ± 0.029	
		1.5	55.4 ± 0.010	
2	n-hexane	3	68.1 ± 0.049	2.5732
		2.5	57.2 ± 0.030	
		1.5	49.8 ± 0.026	
3	Ethyl Acetate	3	74.7 ± 0.005	2.5049
		2.5	67.9 ± 0.004	
		1.5	60.7 ± 0.026	
4	Chloroform	3	67.5 ± 0.029	2.43
		2.5	59.2 ± 0.019	
		1.5	44.9 ± 0.008	

3.2.2 Anti-diabetic activity

Table 3.4: Anti-diabetic activity of C. Reflexa

Sr. no	Sample	Concentration (mg/ml)	Mean ± S.D (% Amylase Inhibition)	IC_{50} mg/ml
1	Methanol	3	80.93 ± 0.026	2.4299
		2.5	76.75 ± 0.027	
		1.5	69.27 ± 0.014	
2	n-hexane	3	78.32 ± 0.014	2.3514
		2.5	72.89 ± 0.025	
		1.5	55.74 ± 0.031	
3	Ethyl Acetate	3	82.20 ± 0.029	2.4406
		2.5	78.17 ± 0.026	
		1.5	71.68 ± 0.033	
4	Chloroform	3	79.56 ± 0.020	2.4128
		2.5	74.8 ± 0.026	
		1.5	65.2 ± 0.025	

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3.2.3 Anti-inflammatory activity

Table 3.5: Anti-inflammatory activity of C. Reflexa extracts

Sr. no	Sample	Concentration (mg/ml)	Mean ± S.D (% Anti-Inflammation)	IC_{50} mg/ml
1	Methanol	3	78.75 ± 0.012	2.5568
		2.5	68.4 ± 0.008	
		1.5	59.75 ± 0.011	
2	n-hexane	3	65.45 ± 0.005	2.4337
		2.5	59.92 ± 0.011	
		1.5	50.48 ± 0.014	
3	Ethyl Acetate	3	80.4 ± 0.021	2.5286
		2.5	70.21± 0.061	
		1.5	60.89 ± 0.052	
4	Chloroform	3	68.2 ± 0.07	2.601
		2.5	60.47 ± 0.04	
		1.5	55.78 ± 0.02	

3.2.4 Hemolytic Activity

Table 3.6: Hemolytic assay of C. Reflexa

Sr. no	Sample	Concentration (mg/ml)	Mean ± S.D (% Hemolysis)	IC_{50} mg/ml
1	Methanol	3	8.98 ± 0.012	2.5244
		2.5	6.58 ± 0.008	
		1.5	4.19 ± 0.011	
2	n-hexane	3	11.97 ± 0.005	2.364
		2.5	10.17 ± 0.011	
		1.5	4.79 ± 0.014	
3	Ethyl Acetate	3	8.78 ± 0.021	2.4748
		2.5	7.18 ± 0.061	
		1.5	5.10 ± 0.052	
4	Chloroform	3	12.79 ± 0.07	2.4543
		2.5	11.57 ± 0.04	
		1.5	9.75 ± 0.02	

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3.2.5 Thrombolytic Activity

Table 3.7: Thrombolytic activity of C. Reflexa methanol extract and its fractions

Sr. no	Sample	Concentration (mg/ml)	Mean ± S.D (% Clot Lysis)	IC_{50} mg/ml
1	Methanol	3	80.5 ± 0.033	2.4288
		2.5	71.14 ± 0.008	
		1.5	54.71 ± 0.033	
2	n-hexane	3	75.8 ± 0.027	2.6901
		2.5	65.25 ± 0.004	
		1.5	48.28 ± 0.027	
3	Ethyl Acetate	3	66.9 ± 0.029	2.5992
		2.5	54.21 ± 0.005	
		1.5	45.62 ± 0.029	
4	Chloroform	3	35.93 ± 0.041	2.5566
		2.5	25.78 ± 0.001	
		1.5	18.14 ± 0.041	

3.2.6 Antibacterial activity

Table 3.8: Antibacterial activity of C. Reflexa extracts

Sr. No.	Strains	DMSO (10%) Zone of inhibition (mm)	Sample	Concentration of sample (mg/ml)	Diameter of zone of inhibition (mm)
1.	Staphylococcus Aureus	00	Methanol	300	26 ± 0.94
				200	21 ± 0.07
				100	19 ± 0.48
			n-hexane	300	22 ± 0.05
				200	20 ± 0.8
				100	18 ± 0.05
			Ethyl acetate	300	23 ± 0.62
				200	21 ± 0.33
				100	20 ± 0.04
			Chloroform	300	22 ± 0.32
				200	18 ± 0.05
				100	16 ± 0.63
2.	Escherichia Coli	00	Methanol	300	20 ± 0.70
				200	18 ± 0.52
				100	16 ± 0.02
			n-hexane	300	19 ± 0.05
				200	18 ± 0.46

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				100	16 ± 0.02
			Ethyl Acetate	300	20 ± 0.40
				200	18 ± 0.03
				100	17 ± 0.35
			Chloroform	300	12 ± 0.05
				200	11 ± 0.02
				100	10 ± 0.06
3.	Klebsiella Pneumoniae	00	Methanol	300	21 ± 0.45
				200	20 ± 0.02
				100	18 ± 0.06
			n-hexane	300	20 ± 0.35
				200	19 ± 0.08
				100	16 ± 0.24
			Ethyl acetate	300	21 ± 0.35
				200	19 ± 0.07
				100	18 ± 0.02
			Chloroform	300	13 ± 0.01
				200	12 ± 0.02
				100	11 ± 0.01

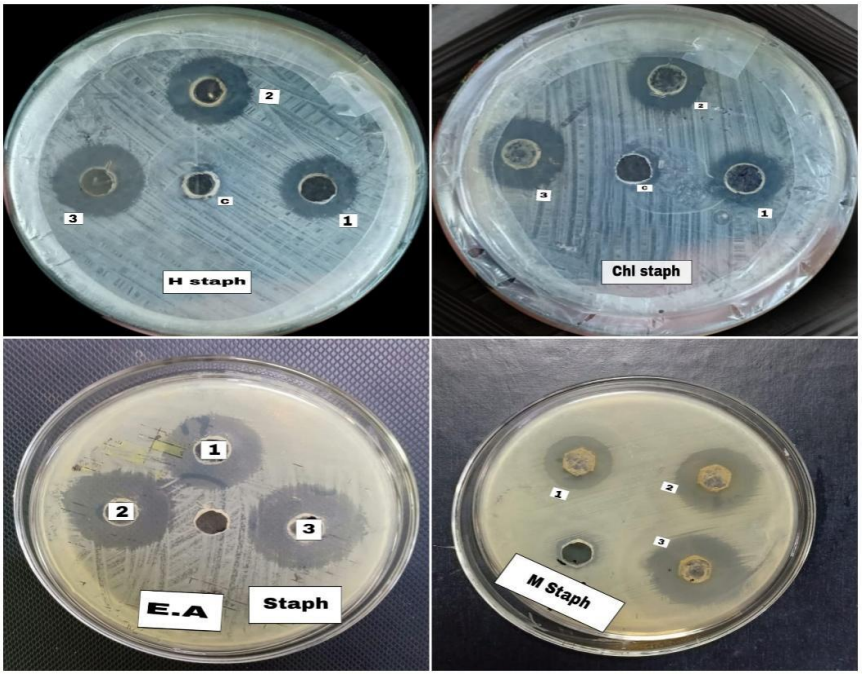

Figure 3.5: Antibacterial effect of C. reflexa methanol extract and its fractions against S. Aureuss

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4. DISCUSSION

It is well recognized that plants are a source of chemicals for the creation of novel medications. Morphine, vincristine, vinblastine, digoxin, reserpine, and aspirin are just of the medications that have been developed from plants. Due to a number of issues, including the emergence of resistance and the adverse effects connected to the use of contemporary medications like antibiotics and anticancer medications, there is a huge interest in medicinal plants today. Many chemical compounds that can be beneficial to the human beings are present in the medicinal plants that are not identified yet. Due to their versatile actions and few side effects, medicinal plants are attracting the attention of most researches looking to evaluate new medications. Since the prehistoric age, one of humans' primary concerns has been the treatment and cure of diseases (Salmerón-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020).

In our research on C. reflexa, analysis of photochemistry confirmed the presence of phenols and tannins in methanol and ethyl acetate extract. Steroids and glycosides were not present in n-hexane fraction. Terpenoids were absent in chloroform fraction. Previous studies also reported the presence of steroids, saponins, and flavonoids in extracts of C. reflexa (Roy et al. 2013; N Sharma, 2021). But alkaloids were reported absent in the extracts i.e. methanol, ethyl acetate, n-hexane, chloroform (Rath et al. 2016; Akhtar et al. 2019). Total phenolic and flavonoid content showed the highest amounts of flavonoids and phenols in the ethyl acetate fraction. Results of TPC were in following order: ethyl acetate (34 ± 0.026 mg GAE/g extract) > methanolic extract (32 ± 0.017 mg GAE/g) > n-hexane (24 ± 0.018 mg GAE/g) > chloroform (23 ± 0.017 mg GAE/g). TFC results order: ethyl acetate (87 ± 0.014 mg QE/g) > methanolic extract (43 ± 0.010 mg QE/g) > n-hexane (42 ± 0.004 mg QE/g) > chloroform (24 ± 0.029 mg QE/g) shown in figure 3.2 and 3.3. Study on this parasitic plant by (Akhtar, Akbar, Erum, & Bhatti, 2019; Sharma, Kumar, & Gupta, 2021) also demonstrated that C. reflexa has a significant amount of total phenols. The presence of higher total phenolic and flavonoid contents in the ethyl acetate formulation may be due to the lower polarity of polyphenols and flavonoids, which make them compatible with medium polarity solvents such as ethyl acetate.

Chemical compounds identified by GC-MS possess several medicinal properties. Identification and quantification of the constituents present in the experimental plant sample was performed by GCMS method. Peak area, molecular formula and retention time were used to confirm the identification of the compounds present in the plant sample. A total of 20 compounds were identified by GC-MS analysis of Cuscuta reflexa methanol extract, representing several medicinal qualities. The TIC curve and different analyzed values of the methanol soluble compounds of C. reflexa is shown in Figure 3.4 and Table 3.2. Following compounds are present in the extract of Cuscuta reflexa which include aromatic hydrocarbons, organic compounds, terpenoids, alcohol, carbohydrate, fatty acids which possess pharmacological properties: methyl 2-O-methyl-alpha-D-Xylofuranoside, 3-O-Methyl-D-fructose, p-xylene, butylated hydroxytoluene, 1,3-dimethyl-benzene, chlorobenzene, methyl 2-O-methyl-alpha-D-Xylofuranoside, 2-Trimethylsilyl-1,3-dithiane, Hexadecanoic acid, 7,10,13-Hexadecatrienoic acid, trans-Geranylgeraniol, Farnesol formate, Bis (2-ethylhexyl) phthalate, 1,4-Benzenedicarboxylic

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acid, bis(2-ethylhexyl) ester, 4-(4-Hydroxy-2,5-dimethylbenzyl)morpholine, 4-(7-Methyloctyl)phenol, decamethyl-tetrasiloxane, 2-(Pyridin-2-ylformamido)acetic acid. These bioactive compounds include: 1,3-dimethyl-benzene: anticancer, anti-bacterial and anti-fungal (Matysiak, 2015), p-xylene: anti-oxidant, anti-fungal, anti-bacterial (Matysiak, 2015), Butylated hydroxytoluene: anti-oxidant (Yehye et al., 2015), anti-viral (Reimund, 1987), 3-O-Methyl-D-fructose: antimicrobial (Jumina, Mutmainah, Purwono, Kurniawan, & Syah, 2019), Methyl 3,5-di-O-methyl alpha-D-Xylofuranoside: antioxidant (Khan, Yusufzai, Kaun, Shah, & Idris, 2016), 2-Trimethylsilyl-1,3-dithiane: antibacterial (Aminah & Tanjung, 2018), Hexadexanoic acid, methyl ester: anti-inflammatory, anti-cancer, hepatoprotective (Singh, Nair, Jain, & Gupta, 2008), antioxidant, hypocholesterolemic (Alghamdi et al., 2018; Siswadi & Saragih, 2021), and anti-diabetic (Rath, Panigrahi, Kar, & Maharana, 2018), 7,10,13-Hexadexatrienoic acid, methyl ester: anti-oxidant, anti-inflammatory, anti-microbial (Ahmed et al., 2022), 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-: anti-inflammatory, anti-arthritic, anti-microbial, anti-tumor, anti-protozal, chemopreventive (Jenecius, Uthayakumaria, Mohan, & Sciences, 2012) cytotoxic, insecticidal (Jenecius et al., 2012), trans-Geranylgeraniol: anti-cancer (Campia et al., 2009; Kusama et al., 2002), anti-microbial (de Wolf et al., 2017), Farnesol formate: anti-obese (Kim et al., 2017) anti-inflammatory, anti-allergic (Ku, Lin, & Medicine, 2015), 4-(4-Hydroxy-2,5-dimethylbenzyl)morpholine: hypolipidemic, hypocholestrolemic, antioxidant (Chrysselis, Rekka, & Kourounakis, 2000), Tetrasiloxane, decamethyl: used in antiperspirant, deodorants, anti-foaming, skin protectants, skin conditioning (Etteieb et al., 2016).

Antioxidant potential of the ethyl acetate fraction (74.7 ± 0.005) was highest at 3 mg/ml, as compared to methanolic extract (69.2 ± 0.096) and other fractions. The value of ascorbic acid radical scavenging activity was 99.6% shown in table 3.3. This experiment was also performed by (Akhtar et al., 2019), their findings showed matching with the results of recent studies, fraction with ethyl acetate displayed the best results that can be due to the highest amount of flavonoids and phenols in the ethyl acetate fraction. Anti-diabetic activity results showed that the ethyl acetate fraction of C. reflexa exhibited the highest percentage of inhibition i.e. 82.20 ± 0.029, followed by methanol extract (80.93 ± 0.026), chloroform (79.56 ± 0.020) and n-hexane (78.32 ± 0.014). On the basis of oral glucose tolerance tests, (Rahmatullah et al., 2010) found that effects of the hypoglycemic methanol extract and chloroform extracts of C. reflexa. The outcomes revealed that rats given extracts of C. reflexa orally, showed considerable hypoglycemic action in response to both methanol and chloroform extracts of C. reflexa. Phenolic and flavonoid chemicals in plant extracts give them their antidiabetic properties (Tanruean, Kaewnarin, Suwannarach, & Lumyong, 2017). Flavonoids, triterpenoids, alkaloids, and phenolics are secondary metabolites due to which a plant shows anti-diabetic property (Kifle, Yesuf, & Atnafie, 2020). The presence of these phytochemicals in C. reflexa extract explains its anti-diabetic activity. Anti-inflammatory activity results showed that ethyl acetate soluble fraction has highest anti-inflammatory activity (80.4 ± 0.021) followed by methanol (78.75 ± 0.012), chloroform (68.2 ± 0.07), n-hexane (65.45 ± 0.005) shown in table 3.5. (Udavant, Satyanarayana, & Upasani, 2012) study on C. Reflexa also showed that methanol extract and its ethyl acetate fraction exhibited significant anti-inflammatory and cytotoxic activities

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than can be explained due to the presence of phenols and polyphenols. Presence of flavonoids in the extracts can relate with their anti-inflammatory activity. Highest amount of flavonoids presence in the ethyl acetate fraction prove its highest anti-inflammatory activity among all other extracts.

In-vitro thrombolytic activity of C. reflexa in this study showed that methanol extract has highest clot lysis activity (80.5 ± 0.033), followed by n-hexane (75.8 ± 0.027), ethyl acetate (66.9 ± 0.029), chloroform (35.93 ± 0.041) shown in table 3.7. In a previous study by (Azad et al., 2020) it was reported that dominant thrombolytic properties were found in the crude ethanol extract (44.63%), chloroform soluble fraction showed 35.93% activity which is similar to our results. Hemolytic activity of the C. reflexa methanol extract and its fractions against normal human erythrocytes showed that hemolytic activity performed on methanol extract and its fractions showed that at the concentration of 1.5 mg/ml extract and all fractions are non-toxic, at the concentration of 2.5 mg/ml and 3 mg/ml, n-hexane (10.17 ± 0.011, 11.97 ± 0.005), and chloroform fraction (11.57 ± 0.04, 12.79 ± 0.07) both are toxic as there hemolytic activity increased at this concentration shown in table 3.6.

Methanolic extract and its fractions exhibited strong antibacterial activity against all tested bacterial strains, including gram positive bacteria (Staphylococcus Aureus) and gram negative (Escherichia coli, Klebsiella Pneumoniae), at 300 mg/ml of concentration shown in table 3.8. We started this experiment with 3 mg/ml of extract, but at e of the extract was showing any inhibition to the bacteria. S at this concentrationo we increased the concentration and at the concentration 100 mg/ml it started to showing visible inhibition zonesctivity was performed using agar well diffusion method and results showed that at 300 mg/ml fractions of C. reflexa exhibited more antimicrobial activity. Against E. coli, S. Aureus, and K. pneumoniae zone of inhibition showed by methanol extract: 20 ± 0.70 mm, 26 ± 0.94 mm, 21 ± 0.45 mm, ethyl acetate: 23 ± 0.6 mm, 20 ± 0.40, 21 ± 0.35 and n-hexane: 22 ± 0.05 mm, 19 ± 0.05 mm, 20 ± 0.35 mm respectively. Chloroform fraction showed 22 ± 0.32 mm of zone against S. aureus and very less antibacterial potential against K. Pneumoniae and E. coli. In case of 10% DMSO, no inhibition zone was observed. Ethyl acetate fraction of C. reflexa showed highest activity against the tested bacteria in the study (Raza, Mukhtar, & Danish, 2015). (Azad et al., 2020) also investigated the antimicrobial activity of ethanolic extract and its sub-fractions, a. Theyscovered compared to a negative control and standard drug, crude ethanolic extract, aqueous soluble fraction, and organic fraction showed reasonable inhibition to growth of the tested bacteria.

5. CONCLUSION

The findings of this study showed that the anti-oxidant, anti-diabetic, anti-inflammatory, and anti-bacterial effects of C. reflexa are due to phenolic and flavonoid components. Methanol extract and its ethyl acetate fraction exhibited high values of phenolic and flavonoid compounds, these extracts found rich in flavonoids and phenolic compounds exhibited highest biological activities. GC-MS analysis of methanol extract of C. reflexa showed 20 different peaks and identified phytochemicals responsible for its biological properties i.e. anti-oxidant, anti-microbial, anti-inflammatory, anti-cancer. ChlThe

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chloroform fraction showed less activity thanher fractions due to less amotocompounds. Chloroform and n-hexane fraction is cytotoxic at 3 mg/ml as their value increased than 10%, they can cause hemolysis against human RBCs. Methanol extract and n-hexane fraction showed high clot lysis percentage at 3 mg/ml. This research results clearly shows thatshoweflexa extract can be used to medications against harmful bacteria, inflammation, diabetes, and oxidation. Additional research is required to find out more active ingredients of this parasitic plant, and investigations on the toxicological profile of this plant should be done. It can further be used for in-silico studies to confirm biological activities of the phytochemicals and drug designing.

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