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**COMPARATIVE EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANT AND  
ANTIBACTERIAL EFFICIENCY OF *CASSIA OCCIDENTALIS* AND *PITHECELLOBIUM  
DULCE* LEAVES EXTRACT**

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**Abstract:**

In the present study, two indigenous plant species of *C.occidentalis* and *P. dulce* are selected and comparative analysis of phytochemicals, antioxidant and anti-bacterial activity of leaves extracts was carried out with different solvents of aqueous, petroleum ether, chloroform and ethanol. The extractive values of these extracts ranged from 3.9-17.5 %w/w and showed the existence of alkaloids, flavonoids, sterols, anthraquinone, cardiac glycosides, tannins and terpenoids of both the plant species. The total phenolic content of both the plant species were in range of 10- 92  $\mu\text{g}/\text{mg}$  and the highest was recorded  $92.45 \pm 1.26 \mu\text{g}/\text{mg}$  in ethanolic extract of *C.occidentalis* and the least value  $10.81 \pm 0.31 \mu\text{g}/\text{mg}$  was observed in the petroleum extract of the *P.dulce*. The TFC are in the range of 9-148  $\mu\text{g}/\text{mg}$  in both plants. The highest value of  $148.99 \pm 6.01 \mu\text{g}/\text{mg}$  was observed in ethanolic extract of *C.occidentalis* and  $79.49 \pm 1.59 \mu\text{g}/\text{mg}$  was recorded in petroleum ether of *P.dulce* respectively. The ethanolic and chloroform extracts showed strong antioxidant and reducing power potentials. Ethanolic and chloroform extracts given the best results in anti-bacterial screening and the MIC were in the range of 200-800  $\mu\text{g}/\text{ml}$  confirming the pharmacognostic feature.

**Keywords:** Phytochemical, antioxidant, alkaloids, ethanolic extracts, antibacterial.

**抽象的 :**

在本研究中, 选择了 *C.occidentalis* 和 *P. dulce* 两种本地植物物种, 并用不同的水溶液、石油醚、氯仿和乙醇溶剂对叶子提取物的植物化学物质、抗氧化和抗菌活性进行了比较分析。这些提取物的提取值范围为 3.9-17.5 %w/w, 表明存在两种植物的生物碱、黄酮类化合物、甾醇、蒽醌、强心苷、单宁和萜类化合物。两种植物的总酚含量在 10- 92  $\mu\text{g}/\text{mg}$  范围内, 最高记录为  $92.45 \pm 1.26 \mu\text{g}/\text{mg}$  在西洋参的乙醇提取物中, 最低值为  $10.81 \pm 0.31 \mu\text{g}/\text{mg}$  在 *P.dulce* 的石油提取物中。两种植物中的 TFC 都在 9-148  $\mu\text{g}/\text{mg}$  的范围内。在西洋参的乙醇提取物中观

察到的最高值分别为  $148.99 \pm 6.01 \mu\text{g}/\text{mg}$ , 在 *P.dulce* 的石油醚中分别记录到  $79.49 \pm 1.59 \mu\text{g}/\text{mg}$ 。乙醇和氯仿提取物显示出很强的抗氧化和还原能力。乙醇和氯仿提取物在抗菌筛选方面的结果最好, MIC 在  $200\text{-}800 \mu\text{g}/\text{ml}$  的范围内, 证实了生药学特征。

**关键词:** 植物化学, 抗氧化剂, 生物碱, 乙醇提取物, 抗菌。

### Introduction:

Natural plant sources have served us with various groups of impressive modern drugs form thousands of years [1]. The use of herbal drugs in modern medical practises is becoming more popular, both in terms of awareness and acceptance. According to WHO, 85-90 % of the world's population relies on plant-based traditional medicines for primary health care [2]. Medicinal plants play an important role in folk medicine and the synthesis of a wide range of biologically active secondary metabolites, many of which have been shown to play a variety of roles in human ailments [3-4]. In recent years, the demand for plant secondary metabolites has increased dramatically in order to better understand various diseases and disorders around the world. They are now important compounds for a variety of therapeutic purposes or as models for the synthesis of valuable drugs [5]. Until recently, much of the research in the pharmacological field was focused on developing new novel compounds that were more stable and could address a wide range of health issues.

The antioxidant efficiency of these medicinal plants has acquired a lot of attention as part of its phytochemical analysis[6]. Antioxidants are substances that the body uses to protect itself from the damage caused by free radicals, the overabundance of which has been linked to a wide range of diseases and disorders [7]. Antioxidants are essential in a variety of pharmacological activities, including anti-aging,

anti-cancer, anti-inflammatory, and anti-microbial activity [8-9]. Natural antioxidant nutrients like Vit-E,  $\beta$ -carotenes, and lycopene have been shown to diminish the risk of lung, prostate, and stomach cancer [10]. The use of natural anti-oxidants and anti-microbial compounds has already been confirmed by various science research communities as a key to unlocking various oxidative stress-related and pathological aspects. Microbial resistance to nearly every antibacterial agent has been documented [11]. This resistance is largely the result of the indiscriminate use of antibacterial drugs, which are commonly used in the treatment of these diseases. Aside from resistance, certain antibiotics have side effects that limit their use. As a result, there is a pressing need to develop new antibacterial agents with limited side effects. Although extensive research and literature reports for *C. occidentalis* and *P. dulce* are currently underway, comparative data on photochemical, anti-oxidant, and anti-bacterial efficiencies is limited. As a result, the current study sought to investigate the phytochemical activities of these plants from the same Fabaceae family.

### Materials and Methods:

#### Plant material collection:

Dr. A. Amurtha Valli, Associate Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, authenticated the plant materials of *Cassia occidentalis* and *Pithecellobium dulce* collected from local village areas of Nellore

district, Andhra Pradesh state. The plant leaves were thoroughly washed, first with tap water and then with distilled water, to remove any debris or dust particles before being shade dried for 10 days. The dried leaves were then ground to a fine powder in a sterilised mixer grinder for 5 minutes before being stored at room temperature in airtight plastic containers until further use.

#### **Leaf Extract Preparation:**

500 g of previously prepared powder was added to 1500 ml of each of the solvents distilled water, petroleum ether, chloroform, and ethanol and left undisturbed overnight at 10°C. The solution was then allowed to stand for 1-2 hours in a boiling water bath with occasional shaking before being left undisturbed for 24 hours. The preparation was filtered through sterilised Whatman No.1 filter paper, and the filtered extract was concentrated using a rotaevaporator under vacuum below 40°C [12-13]. The resulting dried extract was exposed to UV rays for 24 hours before being tested for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4°C until further use [14].

#### **Extractive value determination:**

Under reduced pressure, the filtrate was concentrated on a rotary evaporator. The various extracts obtained with different solvents were weighed, and the percentage yield of the extract was calculated using the method developed by Banso and Adeyemo (2007) [15] with minor modifications.

#### **Phytochemical Screening:**

Various standard protocols were used to analyse the secondary metabolites (phytochemical) constituents present in the various solvent extracts [16-18].

#### **Quantification of Total phenolic content (TPC):**

The total phenolics in the extracts were estimated using a spectrophotometrically [19]. One mL of the sample (concentration 1 mg/mL) was combined with one mL of Folin and Ciocalteu's phenol reagent. After 3 minutes, 1 mL of saturated Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture and diluted to 10 mL. The reaction was kept in the dark for 90 minutes before the absorbance at 725 nm was measured. Gallic acid was used to construct the standard curve (20–100 µg/mL,  $Y = 0.025x + 0.0378$ ,  $R^2 = 0.997$ ), and the results were expressed in µg gallic acid equivalents/mg extract (GAEs).

#### **Quantification of Total flavonoid content (TFC):**

The flavonoid content of the extracts was quantified using a spectrophotometric method [19]. The extract (250 L) (concentration 1 mg/mL) was combined with 1.25 mL of distilled water and 75 L of a 5% NaNO<sub>2</sub> solution. After 5 minutes, 150 L of 10% AlCl<sub>3</sub> solution was added. After 6 minutes, 500 L of 1MNaOH and 275 L of distilled water were added to the mixture to prepare it. The solution was thoroughly mixed, and the absorbance was measured at 510 nm. The standard curve was calculated using (±)-catechin (20–160 µg/mL,  $Y = 0.0006x + 0.0603$ ,  $R^2 = 0.9997$ ), and the results were expressed as g of (±)-catechin equivalents (CEs) per mg of extract.

#### **DPPH radical scavenging activity (RSA):**

Different concentrations of leaf extracts (0.3 mL) were mixed with 2.7 mL of DPPH radical-containing methanol solution. The mixture was vigorously shaken and left to stand in the dark for 60 minutes. The absorbance at 517 nm was used to calculate the reduction of the DPPH radical [20]. Ascorbic acid was used as a standard.

% Inhibition (RSA) =  $[(\text{Abs of the control} - \text{Abs of the sample}) / (\text{Abs of the control})] \times 100$

Where Abs of control denotes DPPH absorbance and Abs of sample denotes DPPH radical + sample extract absorbance. The half maximal inhibitory concentration ( $IC_{50}$ ) values represented the sample concentration required to scavenge 50% of the DPPH free radicals.

#### **Estimation of reducing power:**

The various extract concentrations (1mL) in 1 mL of deionized water were mixed with phosphate buffer (1mL, 0.2M, pH 6.6) and 10%  $K_4 [Fe(CN)_6].3H_2O$  (10mg/ml). For 20 minutes, the mixture was incubated at 50°C. The reaction was stopped by adding 1mL of 100mg/mL trichloroacetic acid ( $CCl_3COOH$ ) to the mixture, which was then centrifuged at 8000 x g for 10 minutes. The absorbance at 700nm was measured after the upper layer of solution (1mL) was mixed with distilled water and  $FeCl_3$  (0.1mL, 1mg/mL), and the absorbance was measured. Ascorbic acid was used as a model [21-22].

#### **Screening of Anti-bacterial activity:**

A total of four bacterial strains (three gram positive and one gram negative strains), *Bacillus megatrium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *E. coli* strains were chosen for the study and were isolated from hospital wound dressing cotton bandages.

#### **Preparation of inoculums:**

Stock cultures were kept at 4°C on nutrient agar slopes. Transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton Broth for bacteria, which were incubated without agitation for 24 hours at 37°C for further study under sterilised conditions. The antimicrobial activity of various extracts was determined using the agar well diffusion assay [23]. In this method, 100 µl of a 24 h old culture of the test organism was inoculated on the agar plates and then spread on the agar surface with a sterilised glass spreader.

After inoculating the test microorganisms for 30 minutes, wells (5mm diameter) were prepared using a sterilised steel cork borer. Four of the five wells in each plate were loaded with 60 µl of test plant extracts (chloroform, ethanolic extracts of *C. occidentalis* leaves and chloroform, ethanolic extract of *P. dulce*). Sixty µl of the standard antibiotic Gentamycin was loaded in the fifth well as a positive control. The plates were then aerobically incubated for 24 hours at 30°C for bacterial test microorganisms and 48 hours at 25°C for fungal test microorganisms. The diameter of the zone of inhibition was measured to determine antimicrobial activity. At cross angles, the diameter (in mm) of the zone of inhibition was measured, and the mean of three independent measurements was taken. By repeating the procedure with the standard antibiotic, the efficacy of the plant extracts was compared.

#### **MIC (Minimum Inhibitory Concentration) Assay:**

Minimum inhibitory concentration (MIC) was determined using the dilution method, as recommended by the National Committee for Clinical Laboratory Standards [24], as the lowest concentration of the test sample that results in complete inhibition of visible growth. For each microorganism species, different concentrations (ranging from 25-1000 µg/ml) of all extracts prepared aqueous and organic solvents. In a 96-well microtiter plate, a stock solution of each active extract was diluted with Mueller Hinton broth to obtain concentrations ranging from 25-1000 µg/ml (with a gap interval of 25µg/ml). Each bacterial strain received a standardised inoculum with an inoculum size of approximately  $5 \times 10^6$  CFU/ml in each well. The experiment also included controls that did not contain plant extracts or bacterial inoculums.

Microtiter plates were then incubated overnight at 30°C. Following incubation, the MIC was determined as the lowest concentration of extract that inhibited visible bacterial growth. All experiments were conducted in triplicate.

### Results and Discussion:

The phytochemical, anti-oxidant capacity and anti-bacterial activity of aqueous, petroleum ether, chloroform, and ethanolic extracts of the leaves *C. occidentalis* and *P. dulce* on common human pathogens were compared in this study. Because these two plant species are members of the same family Fabaceae, there is insufficiency of data in *in vitro* analysis. The plant leaves collected were subjected to phytochemical extraction procedures using the solvents listed in the first step of the study. **Table 1** shows the extractive value of the extracts that were recorded. Out of the four solvents used in the study (aqueous, petroleum ether, chloroform, and ethanolic), aqueous extracts of the two plant sources yielded the highest extractive values of 7.8 and 17.5 (% w/w), respectively. The chloroform extract yielded the lowest results. The overall extractive values in *C. occidentalis* are 3.9-7.8 (% w/w) and 12.9-17.5 (% w/w) in *P. dulce*. This could be due to the presence of a variety of secondary soluble metabolites in the organic solvent mixture. Other organic solvent extracts contained higher moderate amounts of EV as well. The findings are consistent with those of Sambasivam [25]; Isaac [26] on *C. occidentalis* and Sugumaran [27-28] and Katekhaye *et al.*, 2012 [29] on *P. dulce* leaves extracts. In both plant species, preliminary phytochemical analysis of aqueous and organic solvent extracts revealed the presence of alkaloids, flavonoids, sterols, anthraquinones, tannins, and terpenoids (**Table 2 and 3**). This result agrees completely with the data published

previously by Ganga Rao *et al.*, 2018 [30]; Irshad *et al.*, 2013 [31]; Anil, 2017 [32]; and Vedpriya Arya *et al.*, 2010 [33]. Plant phytochemical constituents differ depending on the species, variety, and part of the plant, as well as growth conditions (soil type, water, and temperature) and age. The phytochemistry varies according to geographical regions, seasons, and time of collection, as well as different climatic conditions [34]. The absence of flavonoids and steroids in *Cassia occidentalis* of African species was previously confirmed by Saganuwan and Glumbe *et al.*, 2006 [35], but our results are antagonistic, which could be attributed to different geographical and climatic conditions in India [33]. The presence of various phytochemicals in these plant species will result in a variety of health benefits.

**Table 1: Extractive values of *Cassia occidentalis* and *Pithecellobium dulce* leaves extracts:**

Plant Source	Solvent extracts	Extractive Values (EV) (%w/w)
<i>Cassia occidentalis</i>	Aqueous	7.8
	Petroleum Ether	5.3
	Chloroform	3.9
	Ethanol	7.2
<i>Pithecellobium dulce</i>	Aqueous	17.5
	Petroleum Ether	15.1
	Chloroform	12.9
	Ethanol	14.2

**TABLE 2: Phytochemicals Analysis of leaves of *Cassia occidentalis*:**

S. NO	Phytochemicals	Test's Applied	AQ Extract	PE Extract	CL Extract	ET Extract
1	Alkaloids	a) Mayer's Test b) Dragendorff's Test c) Wagner's Test	–	+++	+++	++++
2	Saponins	Foam Test	–	–	–	–
3	Flavanoids	a) Ferric Chloride Test b) Shinoida's Test	+	+	+	++++
4	Anthraquinones	Borntrager's Test	–	+	++	++
5	Tannins	a) Ferric Chloride Test b) Lead Acetate Test	+	–	–	++
6	Sterols	a) Salkowski's Test b) Liberman-Burchards Test	–	–	–	–
7	Sugars	a) Barfoed's Test b) Molish's Test	++	–	–	++
8	Cardiac Glycosides	a) Borntrager's Test b) Balget's Test	++	–	++	–
9	Lipids and Oils	Saponification Test	–	+	++	+
10	Coumarins	Sodium Hydroxide Test	++	–	–	++
11	Proteins	a) Biuret's Test b) Ninhydrin Test	–	+	+	+

12	Terpinoids	Salkowski Test	–	+++	++	++
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**Table 3: Phytochemical Analysis of leaves of *Pithecellobium dulce***

S. NO	Phytochemicals	Test's Applied	AQ Extract	PE Extract	CL Extract	ET Extract
1	Alkaloids	a) Mayer's Test b) Dragendorff's Test c) Wagner's Test	–	+++	+++	+++
2	Saponins	Foam Test	–	–	–	–
3	Flavanoids	a) Ferric Chloride Test b) Shinoida's Test	++	–	–	++
4	Anthraquinones	Borntrager's Test	–	–	++	++
5	Tannins	a) Ferric Chloride Test b) Lead Acetate Test	–	+	+	++
6	Sterols	a) Salkowski's Test b) Liberman-Burchards Test	–	++	–	+
7	Sugars	a) Barfoed's Test b) Molish's Test	+	++	–	++
8	Cardiac Glycosides	a) Borntrager's Test b) Balget's Test	–	+	++	–
9	Lipids and Oils	Saponification Test	–	+	+	+
10	Coumarins	Sodium Hydroxide Test	–	–	–	–

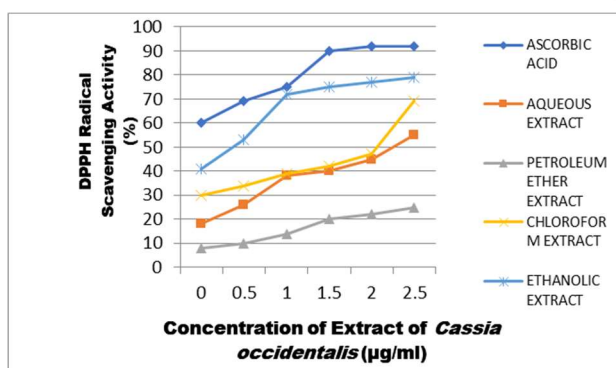
11	Proteins	a) Biuret's Test b) Ninhydrin Test	+	-	-	+
12	Terpinoids	Salkowsk i Test	-	+++	+	+++

The TPC and TFC of *C. occidentalis* and *P. dulce* leaf extracts is investigated (Table 4). The TPC of both plant species was in the range of 10-92 g/mg, with the highest value being  $92.45 \pm 1.26$  µg/mg in the ethanolic extract of *C.occidentalis* and the lowest value being  $10.81 \pm 0.31$  µg/mg in the petroleum extract of *P. dulce*. The chloroform extracts contained moderate to high concentrations. TFC levels in both plants range from 9 to 148 µg/mg (Table 4). The ethanolic extract of *C.occidentalis* had the highest value of  $148.99 \pm 6.01$  µg/mg, while the petroleum extract of *C.occidentalis* had the lowest value of  $9.12 \pm 1.41$  µg/mg. The highest value of  $79.49 \pm 1.59$  µg/mg was found in *P.dulce* ethanolic extract. In terms of extracts, both species followed the same patron of flavonoid concentration. The findings are consistent with previous research by Sharuti Mehta *et al.*, 2010 [7]. Natural antioxidants are primarily composed of phenolic compounds and flavonoids [36]. Phenolic compounds are one of the most numerous and widespread groups of phytoconstituents found in plants [37]. The ability of flavonoids to scavenge hydroxyl radicals and superoxide anion radicals highlights many of the health-promoting functions associated with the prevention of oxidative damage [38]. All of these findings and facts back up the use of these plant species in a variety of medical fields and folk remedies.

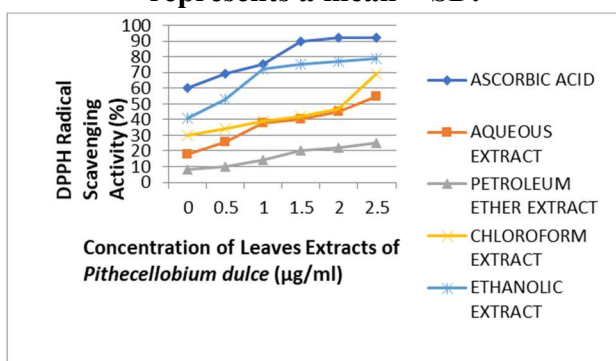
**Table 4: Total phenolic and Flavonoids content of leaves of *C. occidentalis* and *P. dulce*.**

Plant Source	Solvent extracts	Total Phenolic content (TPC) (µg/mg)	Total Flavanoid content (TFC) (µg/mg)
<i>C. occidentalis</i>	Aqueous	$30.15 \pm 0.37$	$30.15 \pm 2.95$
	Petroleum Ether	$17.72 \pm 0.16$	$9.12 \pm 1.41$
	Chloroform	$43.48 \pm 0.37$	$128.01 \pm 2.71$
	Ethanol	$92.45 \pm 1.26$	$148.99 \pm 6.01$
<i>P.dulce</i>	Aqueous	$32.02 \pm 1.22$	$41.20 \pm 1.22$
	Petroleum Ether	$10.81 \pm 0.31$	$17.10 \pm 1.37$
	Chloroform	$57.52 \pm 6.73$	$67.23 \pm 2.73$
	Ethanol	$87.21 \pm 1.59$	$79.49 \pm 1.59$

Fig 1 and 2 shows a significant decrease in the concentrations of DPPH free radicals with increasing concentrations of plant extracts, owing to their ability to scavenge against standard ascorbic acid. The concentrations of an inhibitor required for 50% inhibition of its target (i.e. DPPH radicals) have been calculated as IC<sub>50</sub> values. The scavenging effects of the extracts in both *C. occidentalis* and *P.dulce* plant species were in the order of ascorbic acid > ethanolic extract > chloroform extract > aqueous extract > petroleum extract. The DPPH stable free radical method is a simple, quick, and sensitive method for determining the antioxidant activity of specific compounds or plant extracts [39]. When compared to the standard, the ethanol and chloroform leaf extracts demonstrated a strong response in terms of scavenging activity to the corresponding hydrazine when reacts with the hydrogen donors in the anti-oxidants [40].



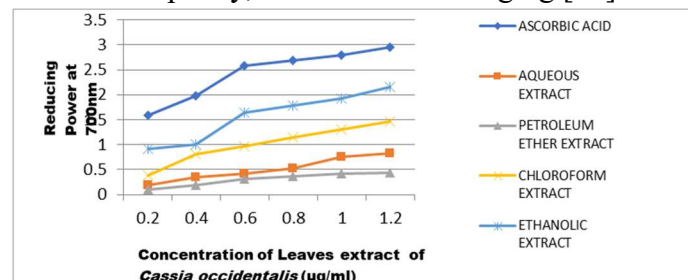
**Fig1: DPPH Free radical Scavenging activities of *C.occidentalis* leaves extracts at different concentrations. Each value represents a mean  $\pm$  SD.**



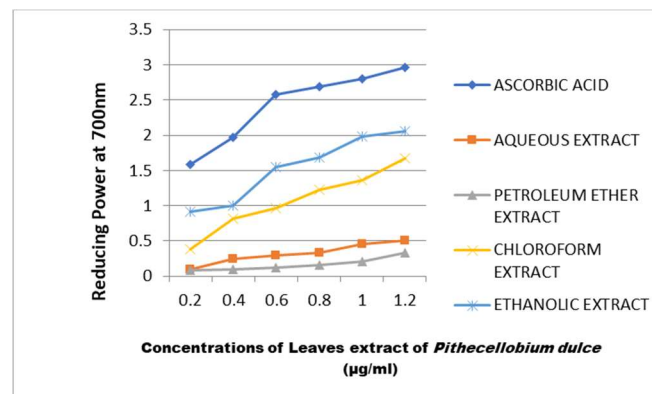
**Fig 2: DPPH Free radical Scavenging activities of *P.dulce* leaves extracts at different concentrations. Each value represents a mean  $\pm$  SD.**

As shown in Fig 3 and 4, the reducing power of both plant leaf extracts increased with increasing concentrations of the various extracts. The graph was used to calculate the  $EC_{50}$  concentrations of extracts that provide 0.5 absorbance at 700nm. The reducing ability of both plant extracts is in the order of ascorbic acid > ethanol extract > chloroform extract > aqueous extract > petroleum extract, which is similar to the order observed in DPPH scavenging activity. The findings and observations made in this study are compared to those made by Mehta *et al.*, 2010 [7] in *C.occidentalis* and Sukantha *et al.*, 2011 [41]; Shanker, 2014 in *P.dulce* (Roxb) [42]. A compound's reducing power capacity can be a

good indicator of its potential antioxidant activity. The antioxidant activity has been attributed to a variety of mechanisms, including chain initiation prevention, transition metal ion catalyst binding, peroxide decomposition, continued hydrogen abstraction prevention, reductive capacity, and radical scavenging [43].



**Fig3: Reducing power activity of *C. occidentalis* leaves extracts in different concentration. Each value represents a mean  $\pm$  SD**



**Fig 4: : Reducing power activity of *P. dulce* leaves extracts in different concentration.**

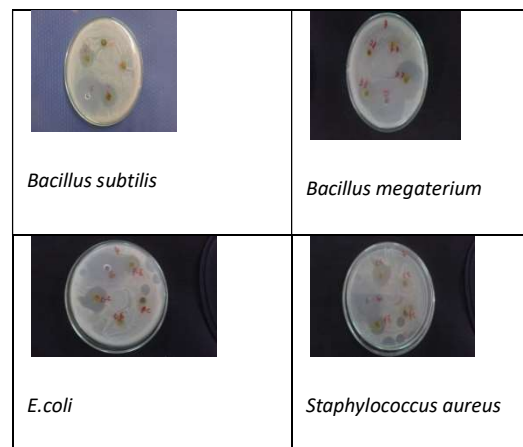
**Each value represents a mean  $\pm$  SD**

Only the chloroform and ethanol extracts of both plants were chosen and tested for anti-bacterial activity against selected human pathogens such as *Bacillus subtilis*, *Bacillus megatrium*, *Staphylococcus aureus*, and *Escherichia coli* using the agar well diffusion method (Figure 5). Gentamicin was the standard antibiotic used in the study, and the results are shown in **Tables 5**. All of the medicinal plant extracts used against pathogenic organisms



demonstrated varying degrees of anti-bacterial activity. When compared to *P. dulce*, chloroform and ethanolic extracts of *C. occidentalis* leaves had a greater inhibitory effect. In both plant species, ethanolic extract was found to be the best organic solvent suitable for antibacterial activity and was recommended. The results showed that chloroform extracts of leaves had a moderate inhibitory effect on selected gram positive and gram negative bacteria. The ethanolic extract of *C. occidentalis* inhibited *Bacillus megatrium* (25mm), *Bacillus subtilis* (10mm), *Staphylococcus aureus* (22mm), and *E. coli* (19mm) (**Table 5; Fig 5**). The chloroform extract of *P. dulce* showed no effect on *Bacillus megatrium*. The findings are consistent with the published data of Mehta *et al.*, 2010 [7]; Taiwo *et al.*, 2013[44] for *C. occidentalis* and Pradeepa *et al.*, 2014 [45]; Sukantha *et al.*, 2014 [46] for *P. dulce*. The MIC was calculated for both extracts and found to be in the 200-800 g/ml range (**Table 6**). The findings of the MIC assay confirmed the findings of the anti-bacterial assay, which revealed that ethanolic and chloroform were potent inhibitors of the pathogens tested [47-48]. The presence of tannins, flavanoids, and phenolic compounds in these extracts contributed to their antibacterial activity. Flavonoids have a wide range of biological activities, including antimicrobial, anti-inflammatory, and cytostatic properties [49]. Antimicrobial activity is induced by compounds that form complexes with bacterial extracellular and soluble proteins. Lipophilic flavonoids can also destroy microbial membranes by exerting pressure on them [50-52]. These phytochemicals have the ability to cross cell membranes, allowing them to enter the cell's interior and interact with intracellular targets critical for antibacterial activity [53]. The

toxicity of phenolic compounds is determined by the number of OH groups present and the location of their occurrence. The increased hydroxylation causes increased toxicity [51,54]. The current study's findings support the traditional use of *C. occidentalis* and *P. dulce* as ethnomedicine.



**Fig 5: Antibacterial activity of ethanolic and chloroform leaves extract on selected human pathogens: C.C: *C. occidentalis* Chloroform extract; C.E: *Cassia occidentalis* Ethanolic extract P.C: *Pithecellobium dulce* Chloroform extract P.E: *Pithecellobium dulce* Ethanolic extract G-Gentamicin.**

**Table 6: Minimum inhibitory concentration of (MIC) values of different leaf extracts of *C. occidentalis* and *P.dulce* on selected human pathogens.**

S. No	Microorganisms	Minimum inhibitory concentrations (µg/ml)			
		<i>Cassia occidentalis</i>		<i>Pithecellobium dulce</i>	
		Chloroform	Ethanol	Chloroform	Ethanol
1	<i>Staphylococcus aureus</i>	250	550	200	250
2	<i>Bacillus megaterium</i>	300	-	375	300

3	<i>Bacillus subtilis</i>	800	450	550	375
4	<i>Escherichia coli</i>	600	200	350	250

### Conclusion:

The tested ethanolic and chloroform extracts of *C. occidentalis* and *P.dulce* were very effective against the tested human pathogens, purification and toxicological studies on these plant extracts, as well as *in vivo* trials, should be conducted so that they can be used as a possible source for the development of a phytomedicine to act against pathogens. Purification of phytoactive compounds and administration of appropriate dosages can improve the anti-bacterial activity of these extracts. As the global landscape shifts toward the use of nontoxic and non-synthetic products, these plant metabolites can be used for traditional medicinal purposes as well as the development of modern drugs that can combat with multi-drug resistant bacterial species.

**Conflict of Interest:** The authors have no conflict of Interest.

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