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Antioxidant and Antimicrobial Activity of Soxhlet Methanolic Seeds Extraction for Five *Cassia* Species

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Abstract
The current study was conducted in the laboratories of the Sciences College/University of Kufa and Al-Ameen Laboratory/Holy Shrine through two aspects, the methanolic extracts for five species of the Cassia genus (C. nodosa, C. surattensis, C.didymobotrya, C. fistula C. gluca) in Iraq during September 2022 - March 2023. As for the biological activity test, it included the methanol extract from the dry seeds using the soxhelt device and the preparation of the concentrations (100,200,300 and 400) mg ml-1. The well diffusion method used to test the evaluation of antibiotics activity against studied bacterial isolates (*Staphylococcus aureus Klebsilla pnemonia. Psedomnus earoginosa, Escherichia.coli).* Bacteria resistant (MDR) to antibiotics (Tetracyclin) and (Cephalexin) were. The results showed the biological effectiveness of the methanolic extract from the seeds of the (CASSIA) plant. It was observed that the best antibacterial activity of the type (*C.fistula*) and a concentration of 400 mg/ml (l) against the bacteria (s. aureus) was the most effective, as the diameter of inhibition was 27 mm, while the least diameter of inhibition of the previously mentioned species extracts was for the type (C.fistula) against the bacteria Klebsilla, where it was zero. The antioxidant activity using the DPPH method at concentrations 100 mg/ml, 0.75 mg/ml. 0.50mg/ml 0.25mg/ml. It also showed that there were significant differences between the concentration of the extract and Cassia spp. There was a strong removal of free radicals at the concentration of 100 mg/ml of C. didomoboteria plant it was 90.206% and the lowest percentage free radicals scavenge was 58.598% at 0.25 mg/ml with C.surinteesis 58.598% at the least significant difference LSD equal to 5.119.

Keywords: Antioxidant, Antimicrobial, Soxhlet Methanolic Seeds, *Cassia* Species

Introduction

Medicinal plants are all higher plants that have been alleged to have medicinal properties, effects that relate to health and used as drugs (KUMAR, 2015). Traditional medicine (TM), variously known as ethnomedicine, folk medicine, native healing, or complementary and alternative medicine (CAM), recently defined as culture-bound method of healing that humans have used to cope and deal with various diseases that have threatened their existence and survival. Several classifications have been attempted for defining and classifying TM (Abdullahi, 2011) [31]. The World Health Organization (WHO) defined TM as the "sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2007). There is an increasing interest in public for consumption of medicinal plants since they are inexpensive and widely available, according to the statics of world health organization, more than 80% of world population particularly in the under- developed countries, provide their primary healthcare necessities from medicinal herbs (Doughari *et al*, 2008) [16]. The history of using medicinal plants to treat diseases goes back to the ancient history. The study of local knowledge about medicinal herbs is becoming increasingly important in defining strategies for conservation and utilization of biological resources (Jeruto *et al*, 2008) [23]. Medicinal plants are extensively utilized in traditional medicine for treating ailments (Davidson-Hunt, 2000) [21]. There are many published reports on the effectiveness of traditional herbs against Gram-positive and Gram-negative microorganisms, and this results in that plants are a good bedrock for modern medicine to treat infectious diseases (Evans *et al*, 2002) [22].There is an increase in resistance of many microorganisms towards established antibiotics, investigation of the chemical compounds within traditional plants has become desirable (Yarmouth, 1991) [24].The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics (Kunin, 1993). Multiple drug resistance has also become prominent due to the common use of commercial antimicrobial drugs which used in the

treatment of infectious diseases, this lead to disease producing are also continuously evolving and speedily becoming resistant to the medicinal options that are available, so the identification of new natural products with antimicrobial activity is one of the ways to resolve this problems (Shoba *et al*, 2014). the hot extract of seeds is taken orally for protection of liver 14. Leaves and seeds are used in the treatment of skin disorders (Ringworm and itch) 6. Stem bark extract is used for various skin ailments, rheumatic diseases and as laxative. In Ayurveda, the plant is used in 'Dadrughani Vati' and 'Pamari Taila' 15. (Yik-Ling 2022). Preparation of plant extracts (Alcoholic extract)

The plants were extracted with methanol of solvent, (alcohol). (Harborne, 1984) [13]. Alcoholic extract was prepared by taking 50gm of powdered sample that was extracted in soxhlet by 200ml of alcohol solvent (Methanol) in flask 500 ml for 24hr then evaporated by oven (40-30)˚C until dried and the extract was kept in refrigerate at 4˚C until used.

Concentration of plants extracts. Stock solution was prepared for extract by dissolving 500 mg of dried extract with 1 ml of distilled water methanol extract, so the final concentration of extract would be 500 mg/ml. From this stock solution at other concentration was prepared 250, 125, 62.5 mg /ml which was used against bacteria (Tripathi *et al*, 2009 [17]; Nwachukwu and Uzoeto, 2010).

Preparation of Chemical Reagents

Alkaloid

1. Dragendroff's reagent:
   This reagent consists of three steps:
a. Solution (A) was prepared by adding 0.6 g of bismuth subnitrate and 2 ml of Hydrochloric acid to 10ml D.W
b. Solution (B) was prepared by adding 6 g of pottasium iodide (KI) to 10 ml of D.W.
c. Solution (A) and solution (B) were mixed, and then 7 ml of HCL was added to the mixture, and then the volume was completed with distilled water to 200 ml and preserve in dark container. (Harborne, 1984) [13].
2. Mayer's reagent:
   Mayer's reagent was prepared by:
a. Dissolving 1.35 g of Mercuric chloride in 60 ml of D.W.
b. Dissolving 5 g of Potassium iodide (KI) in 10 ml of distilled water.
c. Both solutions were mixed and the volume was completed to 100 ml with distilled water (Sousek *et al*, 1999).
3. Benedict's reagent:
   Benedict's reagent was prepared by:
a. Solution (A) was prepared by dissolving 137 gm of sodium citrate and 117 gm of sodium carbonate monohydrate in 700 ml of D.W.
b. Solution (B) was prepared by dissolving 17.3 gm of cupric sulphate in 100 of D.W.
c. Both solutions (A) and (B) were mixed slowly, then the volume completed to 1000 ml by D.W (Al-Khazragi, 1991) [11].

Chemical detection of the active components in plant extracts

Alcohol extract of studied plants were chemically checked for the presence or absence of the following active compounds by treatment with precipitation reagents:-

1. Phenols
a. Ferric Chloride reagents
   Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Audu *et al*, 2007; Obasi *et al*, 2010).
b. Lead acetate
   The extract (50 mg) was dissolved in 5 ml of distilled water. To this, 3ml of 10% lead acetate was added. A bulky white precipitate indicated the presence of phenol compounds (Tamilselvi *et al*, 2012).
2. Alkaloids

Detection by two reagents (Harborne, 1984) [13].

a. Dragendroff reagents

About 1ml of each extract stock was treated with few drops of dragendroff's reagent. The formation of orange coloured precipitate shows the presence of alkaloids.

b. Mayer's reagent

Take five ml of the extract of each plant part were treated with 1 ml of Mayer's reagent.The development of turbidity and white sediment indicate the presence of alkaloids

3. Glycoside

One ml of extract of each part was mixed with 5 ml of Benedict reagent. The appearance of red sediment is indicating the presence of reducing sugar (Keroynz and Anthrykin, 1986).

4. Flavonides

Five ml of each extract was treated with 1 ml of potassium hydroxide alcohol. The development of yellow sediment is an indication of the presence of Flavonides (Harborne, 1984) [13].

5. Saponins

The appear once of foam for a long time as a result of stirring the aqueous solution of plant in test tube indicated saponins existence. (Harborne, 1984) [13].

6. Tannins

Five ml of distilled water was added to 5 ml of extract, the mixture heated at 80-100 °C for 10 minutes in water bath, after being filtered, 5-6 drops of 1 % ferric chloride were added, the dark green color indicates the presence of tannins (Pandey *et al*, 2011).

7. Cumarins

Five ml of plant extract were placed in a test tube and covered by filter paper wetted with sodium hydroxide solution 1 M then put the tube in a boiling water bath for 5 minutes, after which the nomination paper to the UV source, refer color appears greenish yellow at filter paper to positive disclosure (Geisman, 1962).4. Antioxidant Activities, 2-Diphenyl-1-picrylhydrazyl DPPH Radical-scavenging Activity various concentrations of C. surattensis extracts (100, 75, 50, 25) mgLml were mixed with 5.0 rnl of methanolic solution containing DPPH radicals (0.004% w/v). The mixture was shaken vigorously and left to stand for 30 min in the dark (until stable absorbance values were obtained), in a microtiter plate. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = (AOPPH - As)/AoPPH x 100, where as is the absorbance of the solution when the sample extract is added at a particular level and AOPPH is the absorbance of the DPPH solution. Gameli 2022 (Oktay *et al.*, (2003)). Biological effect and Specimens Collection of bacteria: *Staphylococcus aureus*, *Escherichia coli*, *klebsiella* and

pseudomonas were obtained from Central Health Laboratory in AL-Najaf province. The bacteria were activated and sub cultured in nutrient agar and stored on nutrient agar slants at 4 °C.

Preservation: The bacterial isolate was inoculated into the nutrient broth and incubated at 37 °C for 18 hr then the broth culture was preserved by adding glycerol to a final concentration of 20% and stored at -20 °C for 12-18 months (WHO, 2003). Antibiotic Sensitivity Test: A loop full growth from spacemen isolates were inoculated into nutrient broth and incubated at 37C for 24 hr.The bacteria suspension was diluted with normal saline, and compared turbidity with McFarland. Within 15 minutes of adjusting the density of the inoculums, a sterile cotton swab was dipped into the standardized bacterial suspension. The excess fluid was removed by rotating the swab firmly against the inside of the tube above fluid level. The swab was then streaked onto the dried surface of a Mueller-Hinton plate in 2 different planes to obtain an even distribution of the inoculums, and the plates were left for 5 – 15 min at room temperature to dry. With sterile forceps, the selected discs were placed on the inoculated plate and pressed gently into the agar. The plates were incubated at 37C for 18-24 hours in an inverted position. After incubation, the diameters of the complete inhibition zone were noted and measured using reflected light and a ruler. The end point, measured to the nearest millimeter, was taken as the area showing no visible growth. (Harley and Prescott, 1996).

Determination of antimicrobial activity: It was carried out according to disc diffusion method (Chuah *et al*, 2014) the plate of Muller-Hinton agar media was inoculated with Microorganisms (*E.coli, S.aureus, klebsiella* and *pseudomonas*) with sterile swabs. Six mm sterile paper discs made from Whattman No.1 were impregnated with plant extract with different concentrations (62.5, 125, 250, 500) mg/ml. By sterial forceps the discs were position on the inoculated plate and pushed gently into agar. Each plant extract was assayed in triplicate.Sterile paper discs loaded with DMSO were used as negative control.The discs were placed aseptically and distinctively onto the inoculated MHA plates. Agar plates were incubated at 37°C for 16-18 hours. After that, the inhibition zones were measured by ruler (mm).

Statistical Analysis: The experiments were conducted and analyzed as factorial experiments with three replications using a completely Randomized Design (CRD) with two or three factor tested by Least Significant difference (L.S.D).at probability of 1% ($P \leq 0.01$) (Al-Rawi and Kalafallah, 1980).

Results and Discussion

The leaves and seeds of a *cassia* sp. were collected from the province of Babylon / Al-Mahaweel district, after the leaves were washed with sterile distilled water and then it was air dried at room temperature and ground into a fine powder. after which the pods containing the seeds were collected, after that the pods were broken, and the seeds were washed and dried. In the laboratory, 50 grams of leaves were taken and then placed in a crushing container (mortar), after which liquid nitrogen was added to the leaves, after which the amount was crushed with a pulverizer until the amount of nitrogen added evaporated until we get a dry powdered amount from the leaves, after which we weigh the amount.

Plant Sample Collection: The matured pods of *C. surattensis* were collected from Universiti Sains Malaysia (USM), Pulau Pinang, Malaysia. The *C. surattensis* plant (leaves with flowers and pods) was authenticated by a botanist at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia, where a sample with voucher number 11464 has been deposited. The seeds were removed from the pods and were washed under running tap water to remove dirt prior to the drying process. The seeds were dried in an oven at 50°C. Then, the dried seeds were ground into powder and stored in airtight bottles. Seeds were washed with sterile distilled water and then the air is dried at room temperature, weighing an amount of 50 grams of the seeds, and grinding into a fine powder, after which the powder is placed in filter paper, after which it is placed in the extraction apparatus (soxhlet) and 30 ml of methanol is added to it, Samples were crushed and transferred into glass containers preserved it until extraction procedure in the laboratory. 50 gram of ground powders from each plant was soaked in 1000 ml organic solvents i.e., methanol (95% v/v) and With the organic solvent passing over the sample continuously. After one day of dissolving materials were filtered through a Whatman no. 1 filter paper. Then the filters were evaporated using Oven at a temperature of 40 degrees Celsius for seven days. At last, 0.97 g of dried extracts was obtained and then stored atroom temperature room in air tight screw-cap. And the extraction process continues for 24 hours, after which we obtain a mixture of the dissolved substance and the solvent, which is then placed in a glass beaker. After that, the beaker with the mixture is placed in the oven for a week. Now, we get a substance with a gelatinous consistency, with a brown to dark brown color. The material is kept in airtight containers. Weigh 1 microliter of the material obtained with 1 milliliter of sterile distilled water. Mix the sample with a vortex device. We prepare dishes with the culture medium for each selected bacteria. We spread each type of bacteria on each plate.

In Vitro Antioxidant Activity: 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay: DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in solvent. Generally, Methanol and for some cases Ethanol is used as a solvent for DPPH. Ascorbic acid is a strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid is prepared. Different concentrations of the test sample which is to be examined for antioxidant activity is prepared. *viz.* 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml. 3 ml of different concentration of test sample *Cassia glauca* extract was mixed with 1 ml of DPPH solution in dark. 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 1/2 half an hour. When procedure is done than absorbance is taken with the help of U.V. Spectrophotometer at 517 nm (Md. Sikder Almin *et al*., 2010, Molyneux P, 2010).

Antibacterial activity of seed extracts of Cassia sp: The bacterial cultures used in the study were *E. coli, Klebsiella pneumonia, Bacillus subtilus, Salmonella typhi, S. aureus.* These bacteria were provided by Department of Microbiology, Dolphin Institute of Biomedical and Natural Sciences, Manduwala, Dehradun and checked for purity by

convention biochemical methods. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity. Fresh culture were obtained by transferring a loop full of culture into nutrient broth and then incubated at 37ºC overnight. To test antibacterial activity, the well diffusion method was used. The drug (extract) concentration used for antibacterial activity is 200 mg/ml. The microbiological media prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA)

Phytochemical screening: The results of phytochemical screening of methanolic seed methalonic extracts of *Cassia seeds* in table and figure showed high positive reaction with the used reagents. Results indicated the presence of phenolic, alkaloids, glycoside, flavonoid, saponin, tannin and coumarin. A study conducted by Purwar, (2003) using methalonic extract of *Cassia* seeds, reported the presence of alkaloids, flavonoids, phenols, tannins and glycosides Similar study was also conducted by Yadav *et al.*,(2010) reported the presence of secondary metabolism products in all plant parts, variation of compound may differ depending on compound concentration (Jan and Klanus-Heinrich, 2005). The main causes of presence or absence of phytoconstituents in plant extracts depend on solvent polarity, extraction efficiency increases by using different solvents to dissolve the different phytochemical compounds present in different plant parts (Rajan and Dharman, 2014). Gameli *et al* (2016) disagreement in alkaloid and flavonoid were negative in their results in Cassia nodosa also the researchers. *Alam, and El-Nuby* (2022) *were showen the CASSIA FISTULA* disagreement with our study in saponin contain.

Table 1: Preliminary phytochemical screening of *Cassia. spp* seeds extracts. (+) presence compound and (-) for absence. Effect of seeds crude extracts of *Cassia* spp against bacteria *(E.coli*, P earogenosa, Klebseila and *S. aureus*)

M. = Methanol extract of Type of extract						
Plant Reagents		C.didymobotrya	c.gluca	c.nodosa	c.fistula	C.suartteensis
Ferric Chloride	Phenol	+	+	++	+	++
lead acetate		-	-			
Dragendroff reagents	Alkaloid	++	+++	++	+++	++
Mayer's reagent	Alkaloid	++	+++	++	+++	++
Glycoside		++	+	+	+	+
Flavonides		++	+++	+	+++	+
Saponins		++	+	+++	+++	+
Tannins		++	++	+	++	+
Coumarins		+	+	+	+	+

In this study, well diffusion method was used to determine the antibacterial effect of Soxhlet extract method with methanol solvent 96% for seeds extract of *Cassia* spp. The susceptibility pattern to the methanol seeds extract of C.fistula on *S. aureus* was expressed maximum inhibitory zone at concentration 400 mg/ml which was 27 mm, also C.didomoboteria and C.nodosa were gave us 26 mm in same concentration and same bacteria above, then C.gluca and C. Sirateensis which gave the same 25 mm inhibition zone. But in low concentration 100 mg/ml was 12 mm at C. didomoboteria, C.gluca and C. fistula. In P Aeroginosa recorded the inhibition zone diameter to 26 mm in high concentration of methanol seeds extract of C.fistula, it is significant difference with Cassia types. The inhibition zone diameter of K.pneumonia with methanol extract concentration (400mg/ml) of C. didomoboteria, C.gluca, C. nodosa C. fistula and C. sirateensis were 23, 22, 21, 16 and 23 mm respectially didnot differ. The results in table showed bacteria *E.coli* maximum inhibitory zone concentration in methanolic extracts was 25mm in and C.fistula also significant difference on other Cassia spp were inhibitory zone (22mm).The results in same table showed bacteria *S.aureus* in the low concentration(100 mg/ml) of 12mm inhibitory zone (C.didomoboteria, C.gluca and C.fistula) and (C.nodosa and C.fistula) 13 mm inhibitory zone, then the low concentration was 10 mm inhibitory zone of (C.didomoboteria, C.gluca and C.fistula) methlonic seeds extracts at *P aeroginosa* which are not significant difference at 2.26 LSD by use C.didomoboteriain low concentration(100 mg/ml) at last the significant difference in uesing the extract of C.sirateensis was 10 mm inhibitory zone at *P aeroginosa*. The results did not give significant difference among four Cassia types in low concentrations, 10 mm inhibitory zone at K pnuemonia, eccept C.fistula gave us zero inhibitory zone, E. Coli effected by the low concentration of methalonc seeds extract to C.didomoboteria and, C.gluca of inhibition zone (11mm) later C.nodosa 12 mm inhibitory zone then C.fistula 10 inhibitory zone at last zero inhibitory zone of C.sirateensis which is at 2.26 LSD significant difference.

Methanolic extract effect, this is related to the fact that methanol extracts had higher solubility for more phytoconstituents, resulting, the highest inhibition zone. This result is confirmed by the results of type of extract on bacteria *S.aureus* Umagowrie and Chathurdevig (2017) and Al hadad 2017 (Deepak *et al.,* 2013). In a study by Voon *et al*. (2012). (Mortada *et al.,* 2011). This may be due to large number of secondary metabolites compound present in seeds as illustrated in table of function groups recently. The broad-spectrum biological activities of the Cassia seeds extract is possibly due to bioactive substance from this plant that may be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections (Sasidharan *et al*, 2011). Extraction and identification of these phyto-constitutes and determination of their respective antibacterial potencies are helpful to formulate novel

chemotherapeutic agents that should be the future direction for investigation (Doughari *et al*, 2008) [16]. Gumgumjee and Hajar, (2012) the results was agreement with methanolic extract was most effective on both *S. aureus* and *E. coli* bacteria in contrast with hot aqueous and ethanol extract of *C. leptophylla*. Some Cassia spp contain compounds showed inhibition of lipid peroxidation with.This is surprising as the genera are reported to be rich in anthraquinones (Barn, 2012). Agarwal and Paridhavi (2005) found that plant contain a variety of biologically active compounds such as; anthraquinones, flavonoids alkaloids, tannins, saponins, glycosides, terpenoids, steroids and reducing sugars, those have various medicinal properties. Seeds extracts were found to have various activities including protective, antioxidant, Antiulcer and antimicrobial activities (Alam *et al* 2022). The presence of alkaloids, glycosides, saponins, flavonoids and tannins of in *Cassia* seeds extract might beresponsible for the antimicrobial activity and antispasmodic effect (Kishore and Rumana, 2012). These biological activities are related to the molecules structures; by their hydroxyl groups or by. The scientific studies and research on *C. tora* suggests an enormous biological potential of this plant. Clinical and pharmacological studies with standardized extracts and isolated constituents need to be performed to investigate unexploited potential of this plant. There is huge scope for research on *C. tora* and could be further exploited in future as a source of useful phytochemical compound for the pharma industry. There is no doubt that this plant is a reservoir of potentially useful chemical compounds which serve as a drugs, as newer leads and clues for modern drug design by synthesis. It is thought that thorough information as presented in this review on pharmacognosy, phytochemistry and biopotential of *C. tora* may provide strong evidence for the use of this plant in different medicines.

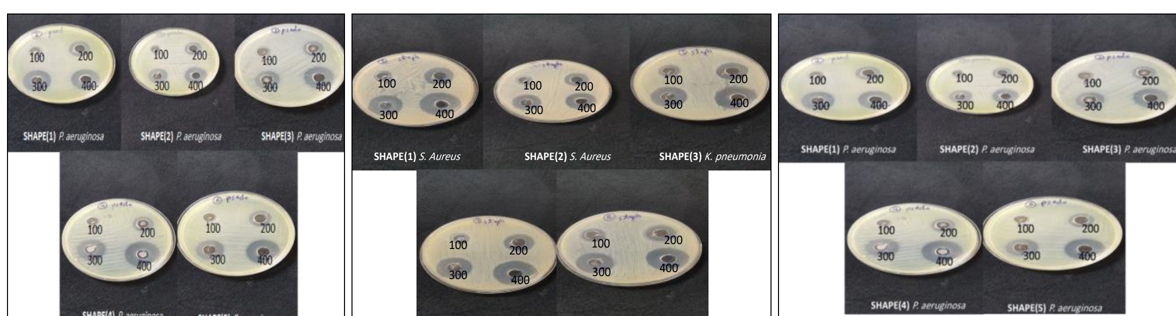

Fig 1: Effect the extract of *C.didomoboteria, C.gluca, C.nodosa, C. fistula and C.sirateensis* against *E.coli, s.aureus* nhibition zone by conc. 100, 200, 300 and 400 mg/ml

Table 2: Interaction between Cassia spp with concentration of methlonic extract from (seeds) in inhibition zone

Cassia spp	Conc. of methanolic extracts	Types of bacteria with inhibition zone (mm)			
		E.coli	***Klebsilla***	***Pseudomonas aeroginosa***	***S aureus***
c.didmobotera	100	11	10	11	12
	200	19	18	16	20
	300	23	21	22	22
	400	25	23	22	26
c.gluca	100	11	10	10	12
	200	15	16	15	18
	300	19	18	18	20
	400	21	22	20	23
c.nodosa	100	12	10	10	13
	200	16	13	14	20
	300	18	17	17	23
	400	22	21	21	26
c.fistula	100	10	Zero	10	12
	200	16	11	15	19
	300	19	15	21	23
	400	25	16	26	27
c.surateensis	100	Zero	10	Zero	13
	200	16	15	15	17
	300	18	21	19	22
	400	21	23	23	25

L.S.D (0.01) = 2.260

The amount of hydroxyl groups found in the phenols are related to their relative toxicity towards microorganisms, with evidence that increased hydroxylation is directly proportion to toxicity (Geissman, 1963). It was also reported that more highly oxidized phenols show more inhibitory activity (Scalbert, 1991). Phenol was also reported that phenolic compound are effective hydrogen donor, making them very good antioxidant (Vinson *et al*, 1998). Also, carboxylic acids were found to be linked with many antimicrobial and antifungal activities which are found to exist in various plant metabolite molecular structures, which had been reported as a strong antibacterial agent (Sultana *et al*, 2010). Many active compounds were produced by plants which contained these active groups (secondary metabolites). Certainly, other chemical components of the extracts could also contribute, although lack of chemical

profiling has never been reported on this. It is possible that these compounds are mainly responsible for the antifungal activities observed in this study.
Against *S. aureus* bacteria at different concentration. The antibacterial activity increases with increasing extract. Antibiotic sensitivity test of *E.coli* and *S.aureus* bacteria: The study of bacterial isolates resistant to antibiotic is one of the most serious medical issues, making it more difficult to selective the best therapeutic drugs (Laxminarayan *et al*., 2013). The dimater of inhibition zones were measured by the ruler in millimeters and compared with (CLSI, 2020). Table (15) and figures (A) and (B) investigated that four types of bacteria study were resistant to the antibiotics tested using disc diffusion method, namely Tetracyclin 10 µg, Cephalexin (CL) 30 µg. Where they mentioned were resistant to the Cephalexin, which was inactive on the isolated bacteria and without inhibition zones, these results were Where mentioned in study that all isolates of *S.aureus* were resistant to oxacillin and sensitive to trimethoprim (Naimi *et al*, 2017) mentioned that *S.aureus* was susceptible to trimethoprim (Chinnambedu *et al*, 2020) mentioned antimicrobial resistance reveals that *S.aureus* was highly resistant to penicillin and cephalexin respectively. Believed that one of the main causes of antimicrobial resistance (AMR) is the wrong or random use of the antibiotics or due to the ability of these bacteria to develop through acquired resistance or transfer of genes or because they have virulence factors or enzymes that work to destroy antibiotics (Fridkin *et al*., 2014). This study has shown development of resistance against antibiotics.The resistance against some antibiotics may occur due to the widespread and often indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases (Adomi, 2006). The resistance of bacteria toward different drug can be due to modification of the target site, bypass of pathways, decreased uptake (reduced intracellular concentration of the antimicrobial agent, either reducing membrane permeability or by active efflux pump), enzymatic inactivation or modification of the drug, or overproduction of the target (Coates *et al*, 2002) or due to the presence of plasmid conferring resistance (Neoji *et al*, 2008).

Table 3: Evaluation of antibiotics activity against studied bacterial isolates

Antibiotics	Con. mg/ml	*E.coli*	*S.aureus*	*Kleibsella*	*P.earogensa*
Tetracyclin		R	R	R	R
Cephalexin		R	R	R	R

The antioxidant activity: The antioxidant activity of Cassia spp seeds extraction by methanol against free radicals in DPPH was determined and has been examined at different concentrations of methanolic extract as well as a control group by using DPPH only. Results in Table found that the methanolic seeds extracts with different species of Cassia used showed strong antioxidant activity with high concentrations 100 mg/ml, 0.75 mg/ml, 0.50 mg/ml and 0.25 mg/ml and shows presence of significant differences among all concentrations compared with the control group for differant Cassia types. Data in table investigated that significant differences with other Cassia types in the highest antioxidant activity was found at C.nodosa and C. fistula which was 85.063% and 80.407% respectively, but the significant differences was abscent among C. didomoboteria, C. gluca and C surateensis (71.871, 72.426 and 70.674) % with LSD equal to 2.473. The interaction in figure showed also that there is siginificant differences among extract concentration and Cassia spp there was strong when using Soxhlet methanol seeds extraction. At concentration 100 mg/ml with C.didomoboteria plant was 90.206% and the lowest the persntage of free ridical scaving was 58.598% on 0.25 mg/ml with C. surinteesis 58.598% in LSD equal 5.119. Result of current study in the same table, were also show significant differences C nodosa in interaction with concentration 0.25mg⁄ml which recorded (81.809%) of free radical cavange percentage or redox potential high enough to scavenge or terminate ROS2, but the concentrations 100 mg/ml of C.gluca C. surinteensis (77.471, 78.556) % although not significant differences among interaction in LSD equal 5.119. (Msaada *et al*., 2013; Anita1 *et al*. 2014), showed many factors can affect their ability in scavenging such as mode of action of natural antioxidants may be varied and could involve multiple mechanism of action,phenols act as primary antioxidants while. The antioxidant activity of a natural source is generally related to either of these activities or as a synergist. Demir and Korukluoglu in (2020) synergism between various antioxidants has been reported, established that coriander antioxidant activity may affected by other factors including production procedures, climatic changes such as average precipitation, harvesting time, altitude, storage conditions significantly influence the composition of phytochemicals in plants which may be not enough in their concentration to give expected effect. Cassia spp seeds were found to possess 3.2% oil content, 45% fatty acids unsaturated/saturated, and tocopherol content (Yadav *et al*, 2010).
Antioxidant Activity in cassia tora the methanolic extract of seeds of C. tora (MECT) shows stronger antioxidant activity. It was found that MECT exhibits stronger antioxidant activity as compared to Alpha-tocopherol. Emodin was demonstrated as antioxidant component of MECT 30. The phenolic active component, alaternin and nor-rubrofusarin glucoside isolated from extract of *C. tora* also showed a potent free radical scavenging activity.

Table 4: The persntage of free ridical scaving antioxident effects *of cassia* sp extract seeds at different concentration and their interaction

Extract (100%)	Plant Type					Meanof Extract
	C.didomoboteria	*C.gluca*	*C.nodosa*	C.fistula	C.sirateensis	
100mg/m	90.206	77.471	87.414	87.969	78.556	84.323
0.75 mg/ml	72.905	72.579	86.333	82.926	73.991	77.747
0.50mg/ml	65.07	70.438	84.696	78.439	71.549	74.038
0.25mg/ml	59.303	69.215	81.809	72.292	58.598	68.243
Mean of Plant	71.871	72.426	85.063	80.407	70.674	
L.S.D. 0.05 Extract = 3.218, Plant = 2.473, Interaction = 5.119						

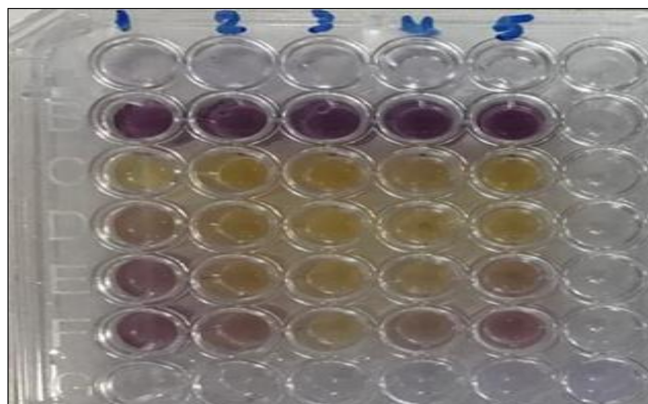

Fig 2: The persntage of free ridical scaving antioxident effects *of cassia* sp extract seeds at different concentration

Conclusions

Cassia spesies seeds vary in their constituent of phytochemicals including phenols, alkaloids, flavonoids, glycosides,tannins, triterpens and coumarins, the antibacterial activity of mathenoilc against G+ve than G-ve, gave us the cassia fistula which showed higher inhabtion zone against MDR of staphlo aureus than the ather type cassia sp**.** All of among cassia sp. In our study the arngment depending on the persentage free ridical scavenge as the folling cassia fistula. The antioxident effects of cassia sp extract seeds at the lowest 0.25 mg ml^{-1} concentration, C. nodosa, C. fistula, C. gluca, C. didomoboteria and C. sirateensis as following.

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