Phytochemical and biological investigation of *Cassia absus* Linn (Stem and Seeds)

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Abstract: Investigation of *Cassia absus* Linn stem and seeds for its phytochemicals afforded eight compounds, hentriacontane, glutinone, physcion, emodin, emodin-6,8-dimethyl ether, xanthorin, ethyl gallate and gallic acid for the first time along with two other reported compounds, chrysophanol and *β*-sitosterol from this plant. Structures of these isolates have been established by various spectroscopic techniques. In present paper the antioxidant and antibacterial activities of various fraction of methanolic extracts from stem and seeds of *Cassia absus* Linn is also reported.

Keywords: Leguminosea, *Cassia absus* Linn, stem and seeds, phytochemicals.

1. Introduction

Cassia absus Linn belongs to the Leguminosae is an undershrub, 10-60 cm tall, widely branched having reddish-yellow flowers and found everywhere in tropics of the world. In Pakistan it is distributed in Khyber Pakhtunkhwa and Punjab provinces where it is locally knows as Chaksu [1]. All parts of *Cassia absus* Linn have wide range of medicinal uses. Roots and leaves are used for the treatment of constipation and tumors respectively [2**]**. Seeds are regarded as enriching the blood as tonic and bitter astringent for the bowels [3] and its paste is eaten to treat anorexia [4]. Seeds are also used in various skin infections, ringworm infestations and in eye diseases such as ophthalmia and conjunctivitis [5]. Literature survey reveals the isolation of *β*-sitosterol [6], gentisic acid, palmitic acid, 5-O-D-glucopyranosyl gentisic acid, ethyl-α-D-galactopyranoside, luteolin, apigenin, hydnocarpin, isohydnocarpin [7], chrysophanol, aloe-emodin, rutin, quercetin, chaksine isochaksine [8], raffinose [9] , 5,7,4′-trihydroxy-8,3-dimethoxyflavone-5-O-α-L-rhamnopyranosyl-7-O-β-D-xylopyranosyl-(1→4)O-β-D-galactopyranoside and 3,5,7,4′-tetrahydroxy-2′,5′-dimethoxy flavone [10], Galactomananan, Stigmasterol, Citric acid, and different phenolic acids and fatty acids [11]. In this paper we report the isolation and identification of hentriacontane (**1**), glutinone (**2**), chrysophanol (**3**), physcion (**4**), *β*-sitosterol (**5**), emodin (**6**), emodin-6,8-dimethyl ether (**7**), xanthorin (**8**), ethyl gallate (**9**) and gallic acid (**10**). Structures of these isolated compounds have been determined by UV, IR, NMR (1D and 2D) and Mass spectral studies.

2. Material and Methods

2.1. General

Silica gel 60 (70-230 mesh ASTM. MERCK), Silica gel 60 F_{254} and Silica gel 60 PF_{256} were used as stationary phase for CC, TLC and preparative TLC respectively and freshly distilled commercial grade solvents as mobile phase. Melting points are uncorrected and determined by Stuart SMP10 melting point apparatus. Ultraviolet absorption spectra were recorded by Shimadzo U-3200 spectrophotometer (λ_{max} in nm). IR spectra were taken on Nicolet-380 FT-IR Spectrometer ($\bar{\upsilon}$ in cm^{-1}). Electron impact (EI) mass spectra were recorded on an AEI MS902 mass spectrometer. ^{1}H-NMR and ^{13}C NMR spectra were recorded in chloroform-d, acetone-d_6 and methanol-d_4 on Bruker AMX-300, 400, 500 and 600 MHz instruments. Chemical shifts (δ), reference to TMS are shown in ppm and coupling constants (J) in Hz. ^{1}H-^{13}C correlations were determined by HSQC and HMBC techniques.

2.2. Plant material

Stem of *Cassia absus* Linn was collected from Boner (Khyber Pakhtunkhwa) and seeds were purchased from local markets of Buner and Peshawar in august 2012. After authentication by plant taxonomists in Department of Botany, University of Peshawar, a voucher specimen (Bot. 20025-PUP) was also deposited in herbarium of the same department.

2.3. Extraction

Air shad dried and pulverized stem (1.2 kg) and seeds (1 kg) of *Cassia absus* Linn were separately extracted with methanol (3×2.5 L) in Soxhlet extractor. The extracts were then concentrated under vacuum in rotary vacuum evaporator.

2.4. Isolation

The crude extracts (stem 166 g and seeds 125 g) obtained were suspended in water and divided further into hexane, dichloromethane and ethylacetate soluble fractions. The dichloromethane soluble fraction from stem (16 g) was subjected to column chromatography eluted with hexane, hexane:ethylacetate and ethylacetate in order of increasing polarity gave twenty two fractions, which were compiled into five different fractions (A-E) after developing and observing their comparative TLC. The fraction A was then purified by column chromatography (hexane 100%) to get **1** (7 mg). Fraction B was purified by column chromatography, eluted with hexane: ethylacetate (9:1) gave **2** (11 mg) and a sub fraction, which was purified by preparative TLC into **3** (17 mg). Fraction C was subjected to column chromatography eluted with hexane:ethylacetate (4:1-7:3) afforded two compounds **4** (28 mg) and **5** (24 mg). Fraction D was divided by column chromatography eluted with hexane:ethylacetate (7:3-3:2) into two sub fractions, which were further purified into **6** (11 mg) and **7** (12 mg) using preparative TLC and hexane:ethylacetate (1:1) as mobile phase. Fraction F was chromatographed over silica gel eluted with hexane:ethylacetate (1:1-2:3) to obtain **8** (9 mg). Dichloromethane soluble fraction (6 g) from seeds was also subjected to column chromatography by above procedure gave three fractions (G-I). These fractions were purified by repeated column chromatography eluting with hexane:ethylacetate (5:1 to 1:4) in order of increasing polarity, afforded three compounds, **5** (18 mg), **9** (14 mg) and **10** (9 mg).

2.5. Antioxidant DPPH assay

Plant extracts were tested for the scavenging effect on DPPH radical according to the method of *Baliyan* et al [12]. Radical scavenging activity of plant extracts and fractions were measured from the bleaching of purple color of DPPH solution in methanol which is due to hydrogen atom donation ability of plant extracts and fractions to the stable DPPH radical. DPPH in methanol (0.1 Mm) was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (10, 20, 40, 60, 80 and 100 µg) and control (without sample). These solutions were kept in dark for 30 minutes and then absorbance was measured at 517 nm. Decrease in absorbance is associated with increase in radical-scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\%\, RSA = \frac{Control\ absorbance\ -\ Sample\ absorbance}{Control\ absorbance} \times 100$$

2.6. Anti-bacterial assay

2.6.1. Test organisms

Two strains of Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and one Gram-negative bacterium, *Klebsiella pneumonia* were used for this activity. These bacterial strains were kept on nutrient Muller-Hinton agar.

2.6.2. Anti-bacterial screening

Agar-well diffusion method was used for anti-bacterial assay [13]. Petri dishes were prepared by pouring 20 ml of molten and sterilized Muller-Hinton agar. These bacterial cultures were spread on the surface of these agar dishes. Wells of 6 mm diameter are bored in each plate. 100 μL of methanol, ethyl acetate, dichloromethane and n-hexane extracts (10 mg/mL) of the seeds and stem

of *Cassia absus* L. were added in each well. 100 μL of streptomycin (2 mg/ml) was used as positive control and 100 μL of DMSO was used as negative control. Plates were then covered and incubated for 24 hours at 37 ^{o}C . Diameter of inhibition zone was measured in millimeters.

3. Results and Discussion

3.1. Phytochemicals

Methanolic extracts from stem and seeds of *Cassia absus* Linn were successively fractioned into hexane, dichloromethane and ethyl acetate soluble fractions. Dichloromethane soluble fractions from stem and seeds were repeatedly chromatographed over silica gel in a column followed by preparative TLC (discussed in section 2.3) and ten compounds (1-10) have been isolated and characterized. Eight compounds (1-8) have been isolated from stem and three compounds (5, 9 and 10) have been isolated from seeds. Compound 5 was isolated from both stem and seeds. These compounds were identified as hentriacontane (**1**) [14], glutinone (**2**) [15,16], chrysophanol (**3**) [17,18], physcion (**4**) [18,19], β-sitosterol (**5**) [20,21], emodin (**6**) [22], emodin-6,8-dimethyl ether (**7**) [23,24], xanthorin (**8**) [25], ethyl gallate (**9**) [26] and gallic acid (**10**) [27] by comparison of experimental physical and spectral data with reported values. Out of all these, eight compounds 1, 2, 4, 6, 7, 8, 9 and 10 were isolated for the first time from this plant.

3.1.1. Hentriacontane (1)

White powder (chloroform); m.p. 66-67 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (6H, t, J = 6.8 Hz, Me-1, Me-31), 1.24 (58H, m, H-2 to H-30); EI MS *m/z*: 436 $[M]^+$ (calcd. 436 for $C_{31}H_{64}$).

3.1.2. Glutinone (2)

Colorless crystals (chloroform), m.p. 244-247 °C; IR υ_{max} cm^{-1}: 2921, 2862, 1706, 1640, 1455, 1384; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.80 (3H, s, Me-25), 0.94 (3H, s, Me-29), 0.97 (3H, s, Me-30), 1.01 (3H, s, Me-27), 1.07 (3H, s, Me-26), 1.14 (3H, s, Me-28), 1.20 (3H, s, Me-23), 1.23 (3H, s, Me-24), 1.26, 1.35 (2H, m, H-19), 1.29, 1.45 (2H, m, H-21), 1.37, 1.41 (2H, m, H-15), 1.39 (2H, m, H-12), 1.40, 1.43 (2H, m, H-16), 1.49, 1.54 (2H, m, H-11), 0.89, 1.58 (2H, m, H-22), 1.60 (1H, m, H-18), 1.62, 1.88 (2H, m, H-1), 1.67 (1H, m, H-8), 1.97, 1.99 (2H, m, H-7), 2.21 (1H, m, H-10), 2.38, 2.43 (2H, m, H-2), 5.69 (1H, t, J = 2.4 Hz, H-6); ^{13}C NMR (75 MHz) δ: 15.65 (C-25), 18.40 (C-27), 19.35 (C-26), 21.58 (C-1), 23.60 (C-7), 24.35 (C-23), 28.25 (C-20), 28.54 (C-24), 30.11 (C-12), 30.34 (C-17), 31.93 (C-15), 32.00 (C-29), 32.36 (C-21), 33.09 (C-30), 34.08 (C-11), 34.52 (C-28), 35.05 (C-9), 35.10 (C-19), 35.94 (C-26), 38.01 (C-2), 38.08 (C-14), 38.91 (C-13), 39.34 (C-22), 43.11 (C-18), 50.00 (C-4), 47.03 (C-8), 50.63 (C-10), 121.35 (C-6), 142.42 (C-5), 215.45 (OC-3).

3.1.3. Chrysophanol (3)

Reddish-yellow crystals (chloroform), m.p. 194-196 °C; IR υ_{max} cm^{-1}; 2924, 1680, 1625, 1434; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s, Me-3), 7.07 (1H, brs, H-2), 7.26 (1H, dd, J = 8.4, 0.9, H-5), 7.62 (1H, brs, H-4), 7.65 (1H, m, H-6), 7.79 (1H, dd, J = 7.5, 0.9, H-7), 11.98 (1H, s, OH-1), 12.09 (1H, s, OH-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.25 (Me-3), 113.73 (C-9a), 115.87 (C-8a), 119.91 (C-5), 121.34 (C-4), 124.35 (C-2), 124.55 (C-7), 133.28 (C-4a), 133.65 (C-10a), 136.94 (C-6), 149.33 (C-3), 162.42 (C-1), 162.72 (C-8), 181.98 (C-9), 192.54 (C-10); EI MS *m/z*: 254 $[M]^+$ (calcd. 254 for $C_{15}H_{10}O_4$).

3.1.4. Physcion (4)

Orange-red crystals (chloroform); m.p. 205-207 °C; IR υ_{max} cm^{-1}; 3332, 3056, 2913, 2848, 1676, 1613, 1562, 1478; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.43 (3H, s, Me-3), 3.92 (3H, s, OMe-6), 6.67 (1H, d, J = 2 Hz, H-7), 7.06 (1H, s, H-2), 7.36 (1H, d, J = 2 Hz, H-5), 7.61 (1H, s, H-4), 12.09 (1H, s, OH-1), 12.28 (1H, s, OH-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 22.12 (Me-3), 56.05 (OMe-6), 106.77 (C-7), 108.19 (C-5), 110.20 (C-8a), 113.20 (C-9a), 121.27 (C-4), 124.49 (C-2), 133.24 (C-4a), 135.28 (C-10a), 148.43 (C-3), 162.51 (C-1); EI MS *m/z*: 284 $[M]^+$ (calcd. 284 for $C_{16}H_{12}O_5$).

3.1.5. β-Sitosterol (5)

White powder (chloroform); m.p. 136-137 °C; υ_{max} cm^{-1}; 3410, 2958, 1640, 1462; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.67 (3H, s, Me-18), 0.78 (3H, m, Me-26), 0.80 (3H, m, Me-27), 0.81 (3H, t, J = 7.6, Me-29), 0.89 (3H, d, J = 5.2, Me-21), 0.99 (3H, s, Me-19), 3.50 (1H, m, H-3), 5.33 (1H, m, H-6) along with other signals; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.97 (C-18), 12.23 (C-29), 18.78 (C-21), 18.98 (C-26), 19.03 (C-27), 19.93 (C-19),71.81 (C-3), 121.71 (C-6) along with other signals; EI MS *m/z*: 414 $[M]^+$ (calcd. 414 for $C_{29}H_{50}O$).

3.1.6. Emodin (6)

Orange-red needles (acetone); m.p. 255-256 °C; IR υ_{max} cm^{-1}; 3479.3, 2955, 1690, 1609, 1562, 1475; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 2.46 (3H, s, Me-3), 6.67 (1H, d, J = 2.5 Hz, H-7), 7.15 (1H, s, H-2), 7.27 (1H, d, J = 2.5 Hz, H-5), 7.58 (1H, s, H-4), 12.07 (1H, s, OH-1), 12.19 (1H, s, OH-8); ^{13}C NMR (75 MHz) δ: 22.5 (Me-3), 106.4 (C-7), 108.60 (C-5), 110.22 (C-8a), 113.21 (C-9a), 122.55 (C-4), 124.05 (C-2), 133.24 (C-4a), 134.95 (C-10a), 148.62 (C-3), 162.40 (C-1), 165.20 (C-8), 166.56 (C-6), 182.15 (C-10), 189.90 (C-9); EI MS *m/z*: 270 $[M]^+$ (calcd. 270 for $C_{15}H_{10}O_5$).

3.1.7. Emodin-6,8-dimethyl ether (7)

Orange-red needles (chloroform); m.p. 211-214°C; IR υ_{max} cm^{-1}; 3430, 2933, 1698, 1633, 1498; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.41 (3H, s, Me-3), 3.97 (3H, s, OMe-6), 4.01 (3H, s, OMe-8), 6.79 (1H, d, J = 2 Hz, H-7), 7.06 (1H, s, H-2), 7.46 (1H, d, J = 2 Hz, H-5), 7.55 (1H, s, H-4), 13.05 (1H, s, OH-1); ^{13}C NMR (100 MHz) δ: 21.95 (Me-3), 56.06 (OMe-6), 56.63 (OMe-8), 103.97 (C-5), 104.76 (C-7), 113.22 (C-9a), 115.12 (C-8a), 120.00 (C-4), 124.81 (C-2), 132.95 (C-4a), 137.74 (C-10a), 146.92 (C-3), 162.66 (C-1), 162.99 (C-8), 165.29 (C-6), 183.01 (C-10), 187.50 (C-9); EI MS *m/z*: 298 $[M]^+$ (calcd. 298 for $C_{17}H_{14}O_5$).

3.1.8. Xanthorin (8)

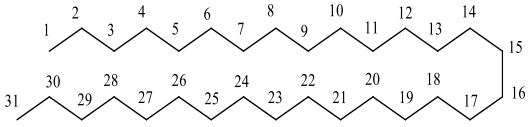
Red crystals (chloroform); m.p. 251-253^{o}C; IR υ_{max} cm^{-1}; 3434, 1690; ^{1}H NMR (400 MHz, CDCl3) δ: 2.45 (3H, s, Me-3), 3.99 (3H, s, OMe-6), 6.66 (1H, s, H-7) 7.09 (1H, br.s, H-2), 7.68 (1H, br.d, J = 1.6 Hz, H-4), 12.27 (1H, s, OH-1), 12.78 (1H, s, OH-8), 13.56 (1H, s, OH-5); EI MS *m/z*: 300 $[M]^+$ (calcd. 300 for $C_{16}H_{12}O_6$).

3.1.9. Ethyl gallate (9)

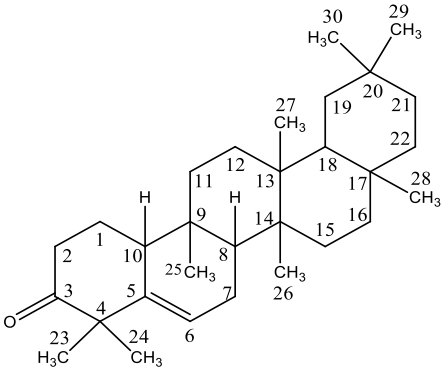
White powder (methanol); m.p. 155-157 ^{o}C; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.33 (3H, t, J = 7 Hz, H-2′), 4.27 (2H, q, J = 7 Hz, H-1′), 7.03 (2H, s, H-2, H-6); ^{13}C NMR (125 MHz) δ: 14.60 (C-2′), 61.67 (C-1′), 110.01 (C-2, C-6), 121.79 (C-1), 139.69 (C-3, C-5), 146.49 (C-4), 168.56 (COO).

3.1.10. Gallic acid (10)

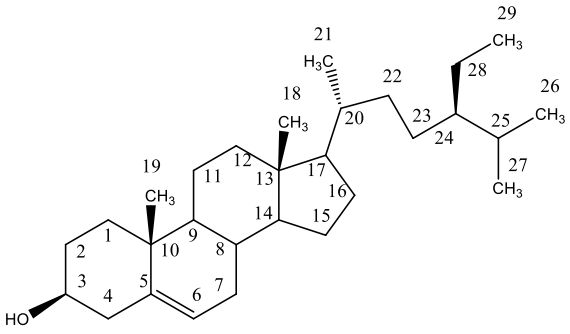
White crystals (methanol); m.p. 250-251 ^{o}C; ^{1}H NMR (600 MHz, CD_3OD) δ: 7.94 (2H, s, H-2, H-6); ^{13}C NMR (125 MHz) δ: 108.68 (C-2, C-6), 120.52 (C-1), 137.93 (C-3, C-5), 145.38 (C-4), 167.51 (COO).



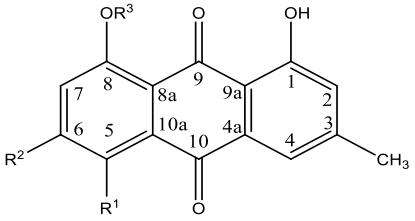
Hentriacontane **(1)**



Glutinone **(2)**



β-Sitosterol **(5)**



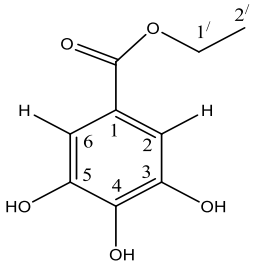
$R^1 = R^2 = R^3 = H$ – Chrysophanol **(3)**

$R^1 = R^3 = H$, $R^2 = OCH_3$ – Physcion **(4)**

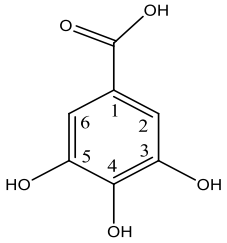
$R^1 = R^3 = H$, $R^2 = OH$ – Emodin **(6)**

$R^1 = H$, $R^2 = OCH_3$, $R^3 = CH_3$ – Emodin-6,8-dimethyl ether **(7)**

$R^1 = OH$, $R^2 = OCH_3$, $R^3 = H$ – Xanthorin **(8)**



Ethyl gallate **(9)**



Gallic acid **(10)**

Fig. 1: Structures of compounds **1-10.**

3.2. Antioxidant DPPH Assay

DPPH radical scavenging activity of various fractions of *Cassia absus* Linn are given in given in Fig. 2. Result shows that amoung all fractions, the methanolic fraction of stem (MSt) shows good degree of free radical scavenging activity. Methanolic fraction obtained from seeds (MSd) of *Cassia absus* Linn also shows considerable radical scavenging activity at low and high concentration. All other fraction also shows considerable radical scavenging activity.

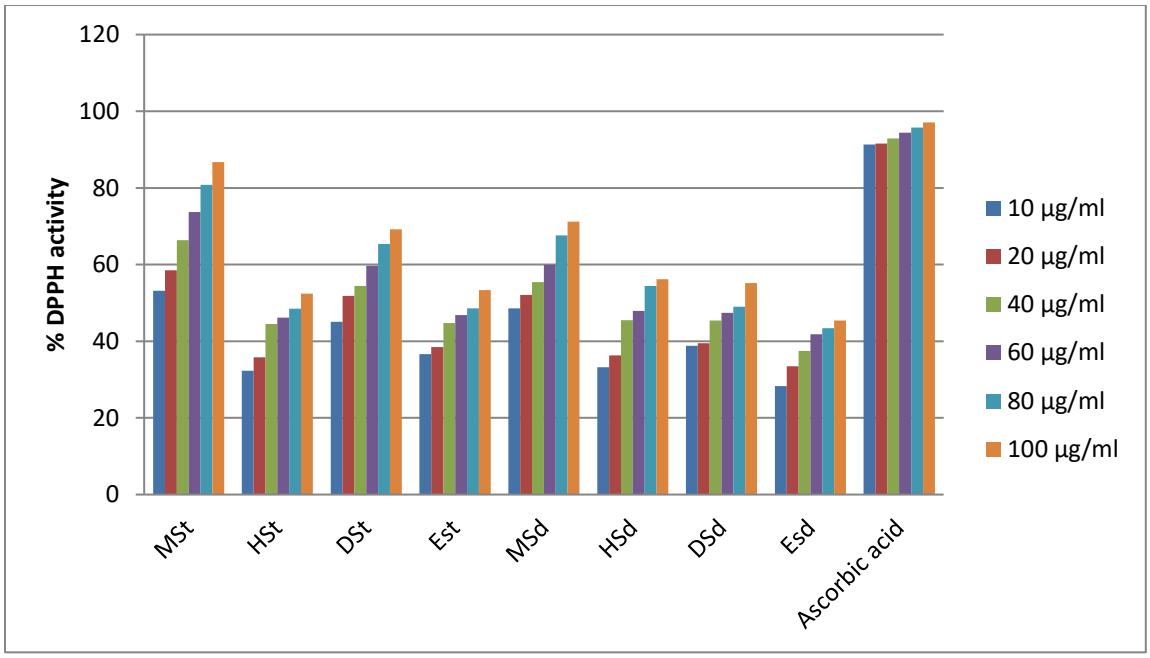


Fig. 2. % DPPH Radical Scavenging Activity of various fractions of Cassia absus Linn stem and seeds.

2.4. Antibacterial activity

Various extracts of *Cassia absus* Linn were evaluated for their antibacterial potential. Results show (Fig. 3) that different fraction good antibacterial activity ranging from ranging from 10 to 18 mm zone of inhibition. Streptomycin was used as standard. Methanol fraction obtained from seeds (MSd) was found to be the most effective against *Bacillus subtilis*, whereas from stem (MSt) was very active against *Klebsiella pneumonia.* Hexane fraction from stem (HSt) and seeds (HSd) show good activity against *Bacillus subtilis* and *Klebsiella pneumonia* respectively. Dichloromethane fraction from stem (DSt) and seeds (DSd) of *Cassia absus* Linn show considerable amount of activity against *Staphylococcus aureus* and *Klebsiella pneumonia* respectively.

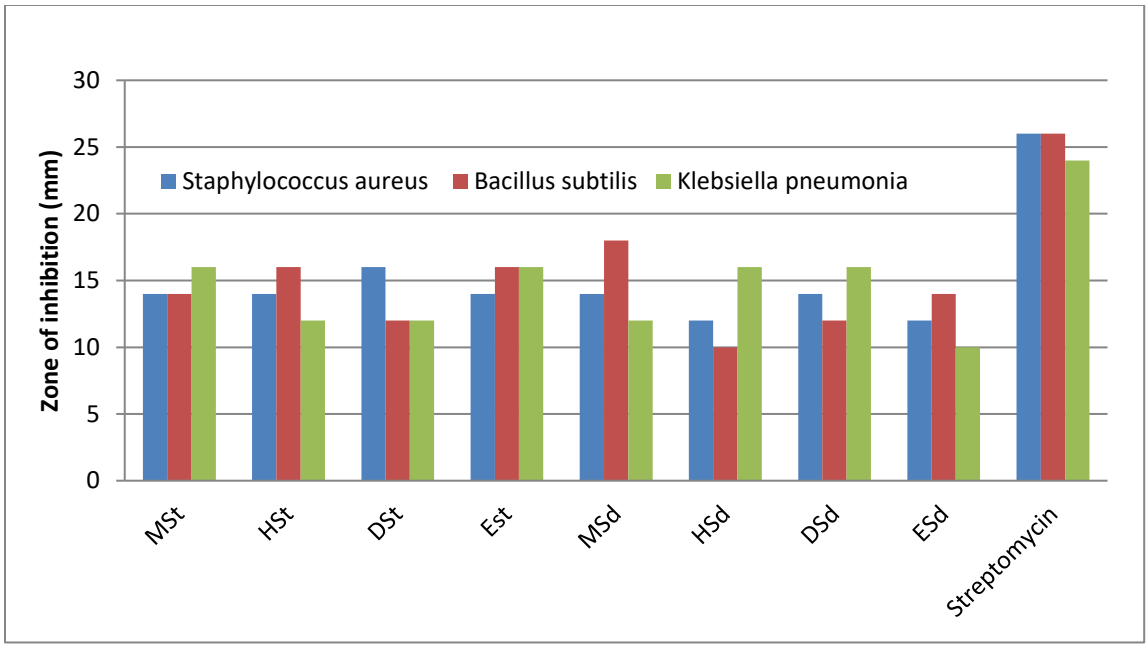


Fig. 3. Antibacterial activity of various fractions of *Cassia absus* Linn stem and seeds.

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Conflict of interest: The authors declare no conflict of interest.
Ethical approval: Not applicable
Data availability statement: The datasets generated and analyzed during the current study are available from the corresponding author on request.

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