Research In Pharmaceutical Biotechnology Vol. 3(7), pp. 85-92, July 2011
Available online at http://www.academicjournals.org/rpb
ISSN 2141-2324 ©2011 Academic Journals

Full Length Research Paper

Phytochemical and biological study of *Striga hermonthica* (Del.) Benth callus and intact plant

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Accepted 08 June, 2011

Preliminary phytochemical screening of *Striga hermonthica* was carried out to assess the chemical contents and biological activity of callus comparing to that of intact plant (upper and underground parts). The results show the presence of terpenes, tannins, coumarins, cardiac glycosides, flavonoids, saponins, anthracenosides and alkaloids. Further, ethanol extracts analysis-using thin layer chromatography (TLC) revealed differences in chemical constituents between calli, and different parts of the plant with five fractions in callus, three fractions in upper-parts and two fractions in underground-parts based on solvent systems. Antimicrobial assay of S*. *hermonthica* extracts revealed various activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* using agar well-diffusion method. The richest extract with phytochemical constituents and most effective one was the ethanol extracts of the different parts.

Key words: *Striga hermonthica*, phytochemical screening, thin layer chromatography, callus, flavonoids, antimicrobial assay.

INTRODUCTION

Striga hermonthica (Del.) Benth (Scrophulariaceae) is an ubiquitous hemi-parasitic plant growing in wide spectrum of food crops, e.g., rice (*Oryza sativa* L.), millet (*Pennisetum glaucum* L. Leeke), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) roots (Tarr, 1962; Hutchinson and Dalziel, 1963; Carson, 1988; Press et al., 2001). It is widespread in West and East Africa (Mohamed, 1994; Mohamed et al., 2001; Musselman et al., 1991). *S. hermonthica* is a well-known medicinal plant that has been used widely in folkloric medicine in some parts of Africa (Choudhury et al., 1998; Kokwaro, 1976; Atawodi et al., 2003). It has a wide range of medicinal uses; the pharmacological abortificient effect, dermatosis, leprosy ulcer, pneumonia and jaundice remedy, trypanocidal effects, antibacterial and anti-plasmoidal activities have been reported (Choudhury et al., 1998; Hussain and Deeni, 1991; Kokwaro, 1976; Nacoulma, 1996; Okpako and Ajaiyeoba, 2004). The plant has also revealed antioxidant property due to its diverse content of phenolic compounds, e.g., luteolin, apigenin, anthocyanins and tannins (Chouldhury et al., 2000; Khan et al., 1998; Kiendrebeogo et al., 2005). *In vitro* propagation of plants holds tremendous potential for the production of high-quality plant based on medicines (Murch et al., 2000). However, the medicinal effectivity of this plant has been studied extensively; nevertheless, none of these reviews has attempted to harness the advantages of *in vitro* propagation and subsequent phytochemical screening for medicinal purposes. In the current study, we successfully induced the callus of *S. hermonthica* using an entire *in vitro* system. The preliminary phytochemical screening of this plant indicated the presence of saponins, tannins, flavonoids, volatile oils and cardiac glycosides (Okpako and

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Ajaiyeoba, 2004). In this contribution, we have initially screened the *in vitro* produced calli of this plant for its phytochemical constituents in compare to phytochemicals of different parts of the intact plant. We have also studied very initially the antimicrobial activity of various extracts of *S. hermonthica* against several standard microorganisms.

MATERIALS AND METHODS

Plant Materials and Microbial Growth Condition

The intact plant was collected from Shambat Forages Field, Khartoum, Sudan. The samples were divided into upper (shoot) and underground (haustoria) part and air-dried at room temperature (25°C). *S. hermonthica* callus was produced using a standard method (Zhou et al., 2004). The calli of different ages of weeks four, six and eight were collected, freeze-dried and stored at - 80°C till used. Petroleum ether, ethanol and water extract were used for phytochemical screening following the standard method (Vaghasiya and Chanda, 2007). *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923 and a fungal strain *Candida albicans* ATCC7596 were obtained from the Department of Microbiology, Institute of Medicinal and Aromatic Plants, National Centre for Research, Ministry of Science and Technology, Khartoum, Sudan.

Phytochemical screening

The presence of triterpenes, sterols, tannins, coumarins, cardiac gylcosides, flavonoids, saponins, anthrocenosides, carotenoids, glycosides and alkaloids were appraised was conducted following the standard protocols (Culei, 1989; Harborne, 1973; Trease and Evans, 1989).

Thin layer chromatography technique

Frozen dry callus of different ages, upper and underground parts of *S. hermonthica* were defrosted and 250 mg extracted with 2.50 ml of 75% ethanol in water bath for 2 h with intermittent cooling every 30 min to avoid decomposition of components. The extracts were transferred to micro-tubes and centrifuge (6000 rpm) for 3 min. Aliquots (5 µl) of the respective solution was applied into Silica gel 60_{254} aluminum plates (Merck, Germany) in four different solvent systems with different polarity (well known for flavonoids separation), Ethyl acetate/Formic acid/Acetic acid/Water (100:11:11:27), Ethyl acetate/Methanol/Water (100:13.5:10), Ethyl acetate/Formic acid/Water (68:8:8) and Butanol/Acetic acid/Water (100:25:125) (Males et al., 2006; Wagner and Bladt, 1996). The experiments were conducted in pre-saturated thin layer chromatography (TLC) chamber. The TLC plates were air-dried at room temperature for 30 min and visualized under 365 nm UV-lamp (UVP, Upland, CA 91786, U.S.A) before and after treating with the detecting reagent (iodine vapor) (Jork et al., 1994). The retention factor (R_f) for the separated spots was calculated using the following equation:

R_f = [Distance traveled by the sample / Distance traveled by the solvent]

Antimicrobial activity

The well diffusion method (Bauer et al., 1966; Schillinger and Lucke, 1989) was used to test antimicrobial activity of S. *hermonthica* extracts. Mueller-Hinton Agar medium was pre-swabbed with tested microorganisms and four wells in each plate were made using cork-borer of 0.6 mm diameter. The extracts at final concentration 2 mg/ml and positive control Kanamycin were used at concentration of 50 µg/ml, with 50 µl per well for each. For the native control solvents alone were applied following the same protocol. The cultures were incubated at 35°C for 24 h and the zones of inhibition were measured. The experiment was done in triplicates and the means of inhibition zones were calculated manually.

RESULTS AND DISSCUSION

Preliminary phytochemical screening

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care (WHO, 1978). *S. hermonthica* is one of these plants, which plays a vital role in the folk medicine in some parts of Africa (Keindrebeogo et al., 2005). Phytochemical screening of *S. hermonthica* callus and intact plant was carried out to appraise the scientific aspects of the traditional uses of this plant. The preliminary screening revealed the presence of various active principle metabolites; these are alkaloids, flavonoids, coumarins, cardiac glycosides, anthracenosides, saponins, tannins, reducing compounds, terpenes and steroids (Table 1). To some extent these results are consistent with Okpako and Ajaiyeoba (2004), who reported the presence of saponins, tannins, flavonoids, volatile oils and cardiac glycosides of the intact plants. In contrast, this is the first reports for coumarins and anthracenosides. The data obtained during this study revealed that *S. hermonthica* callus and intact plant shown the presence of flavonoids, terpenes, sterols and glycosides in approximately high amounts. Kiendrebeogo et al. (2005) estimated the flavonoids content of the *S. hermonthica* acetone aqueous extract to be about 4% mainly as luteolin flavonoid. However, tannins, coumarins, cardiac glycosides, reducing sugar and alkaloids were found in various levels among the tested parts (Table 1). Anthracenosides and emodols were not detected among all tested parts, except in underground (haustoria) parts, which revealed the presence of anthracenosides in trace amount. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to exploring novel compounds. Furthermore, these preliminary investigations facilitate the subsequent quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc (Hassan et al., 2004). *S. hermonthica* has been used extensively in folkloric remedies of many diseases, such as malaria,

Table 1. Phytochemical constituents of *S. hermonthica* different parts of intact plant and callus.

Phytochemical constituents	Screened plant parts		
	Aerial part	Underground parts	Callus
Triterpenes / sterols	++	+	+
Tannins	+	±	±
Coumarins	−	+	+
Cardiac glycosides	+	+	±
Flavonoids	++	+	++
Saponins	++	+	+
Anthracenosides	−	±	−
Emodols	−	−	−
Carotenoids	±	−	−
Glycosides	+	+	+
Reducing compounds	+	+	+
Alkaloids			
-Wagner's test	−	+	+
-Mayer's test	±	+	±

++ = reasonable amount, + = moderate amount, ± = trace amount, − = not detected. These amount remarks estimated by observing the test color intensity which was used as indicative for the phytochemical quantity.

trypanosomasis, and some bacterial infections (Okpako and Ajaiyeoba, 2004). The wide spectrum curative ability of this plant may be due to the diverse phytochemicals that have been reported in this plant (Keindrebeogo et al., 2005; Okpako and Ajaiyeoba, 2004). Nonetheless, still there is shortage in the scientific information about the phytochemical composition of *S. hermonthica* and its active ingredients. Claims in this respect to the therapeutic success of the plant still need more research to prove the scientific usage of this plant in remedies. No attempt has been made by any worker to purify the compounds contained in the plant extract; however, the plant crude extract proved its effectiveness against many diseases (Kokwaro, 1976; Atawodi et al., 2003).

Thin layer chromatography

For further investigation on the chemical constituents of *S. hermonthica* callus and intact plant, a thin layer chromatography screening of richest extracts (alcohol extracts) was carried out using different solvent systems. Since phenolic compounds, e.g., flavonoids were observed in all of the *S. hermonthica* analyzed materials (Table 1). Flavonoids were selected for further analysis using TLC technique for more comparative study between *S. hermonthica* calli and the different parts of the intact plants. TLC plates showed different results illustrated in the figures of TLC chromatograms (Figures 1A to C). Solvent system I (system I hereafter) revealed the best results, followed by solvent system II. System I separated five clear spots from all callus extracts of different ages and three and two spots from the haustoria and shoot respectively (Tables 2 to 5). The spot with Rf ~0.54 showed similarity between all parts of *S. hermonthica*, these similar spots suspected to be chemically identical compounds. In addition, there was a high similarity between chemical constituents of upper parts (shoot) and callus tissues of different ages except in one fraction with Rf ~ 0.24, which appear in the calli extracts and it is not in shoot extract. We observed that the calli tissues of different ages revealed no differences on the TLC chromatograms, which contained five spots with R_f values ~0.24, 0.38, 0.47, 0.57 and 0.94 and thus we found no indication of age impact on the chemical constituents of the calli. Lack of comparative studies made it difficult to assign these separated spots to any of the well-known reference phenolic compounds; however, one of these separated compounds is likely to be luteolin, apigenin, flavone and/or anthocyanins, which was found in high amount (Choudhury et al., 2000; Khan et al., 1998; Kiendrebeogo et al., 2005). The same results of TLC chromatogram were obtained in solvent system II with few differences in number of separated spots in each plant parts. Solvent systems III and IV seem to be not suitable, which separated unclear, less and diffusing-like spots (Figure 1A). Generally, the spots were unclear in most of the preparations and appeared pale yellow, yellowish green and green in some when visualized by eye, however, under UV-lamp in long wave length 365 nm the spots colors were fluorescent blue with exception of the spots of calli and shoot with R_f values ~0.94, 0.95, 0.91 and 0.97 gave orange to red fluorescent color under 365 nm UV-lamp (Figure 1C). The similarity of shoot and calli of *S. hermonthica* in most of their flavonoids components suggests that the callus is a possible

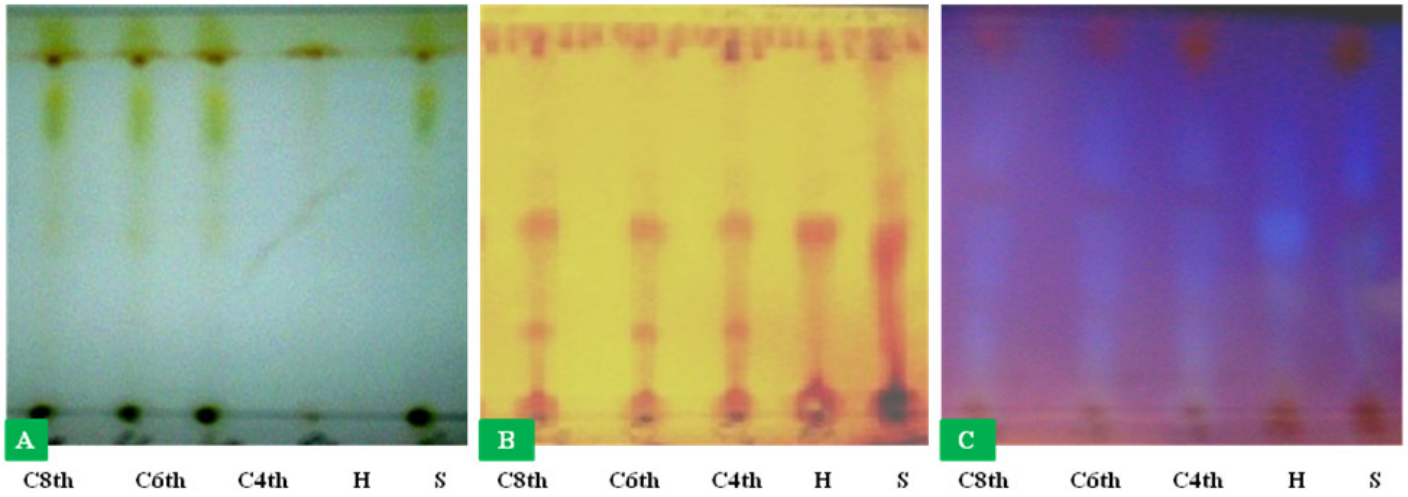

Figure 1. TLC chromatograms of alcoholic extracts of *S. hermonthica* (C4th, C6th and C8th represent calli of week 4, 6 and 8, respectively. H = haustorium part, S = shoot); (A) TLC chromatogram developed on system IV before treating with iodine vapor; (B) TLC chromatogram developed on system I, spots color after treating with iodine vapor; (C) TLC chromatogram of system I under UV-lamp of long wavelength 365 nm.

Table 2. TLC remarks of alcoholic extract components of *S. hermonthica* developed on system I (Ethyl acetate/formic acid/Acetic acid/Water, 100:11:11:27).

Plant parts	No. of spots	R_f (values)*	Separated spots remark	Spots under UV-lamp at (365nm)
Shoot	1	0.26	Pale yellow green/-	Blue fluorescent
	2	0.39	Pale yellow green/-	Blue fluorescent
	3	0.97	Green	Orange/Red fluorescent
Haustoria	1	0.57	Pale yellow/-	Blue fluorescent
	2	0.91	Pale yellow/-	Blue Fluorescent
Callus of week (4)	1	0.24	Pale yellow green/-	Blue fluorescent
	2	0.38	Pale yellow green/-	Blue fluorescent
	3	0.47	Pale yellow/-	Blue fluorescent
	4	0.57	Pale yellow/-	Blue fluorescent
	5	0.94	Green	Orange/Red fluorescent
Callus of week (6)	1	0.26	Pale yellow green/-	Blue fluorescent
	2	0.38	Pale yellow green/-	Blue fluorescent
	3	0.48	Pale yellow/-	Blue fluorescent
	4	0.57	Pale yellow/-	Blue fluorescent
	5	0.95	Green	Orange/Red fluorescent
Callus of week (8)	1	0.26	Pale yellow green/-	Blue fluorescent
	2	0.39	Pale yellow green/-	Blue fluorescent
	3	0.47	Pale yellow green/-	Blue fluorescent
	4	0.57	Pale yellow green/-	Blue fluorescent
	5	0.95	Green	Orange/Red fluorescent

*Values represent Means calculated from triplicates experiments. (-) means these sports are almost invisible in some.

alternative source for the production of the phytochemical constituents. The results suggest further studies in this field.

Antimicrobial activity of *Striga* extracts

The antimicrobial effects of medicinal plants are well

Table 3. TLC remarks of alcoholic extract components of *S. hermonthica* developed on system II (Ethyl acetate/Methanol/Water; 100:13.5:10).

Plant parts	No. of spots	R_f (values)*	Separated spots remark	Spots under UV-lamp at (365nm)
Shoot	1	0.49	Pale yellow green/-	Blue fluorescent
	2	0.56	Pale yellow green/-	Blue fluorescent
	3	0.94	Green	Orange/Red fluorescent
Haustoria	1	0.54	Pale yellow/-	Blue fluorescent
	2	0.93	Pale yellow/-	Blue fluorescent
Callus of week (4)	1	0.24	Pale yellow green/-	Blue fluorescent
	2	0.54	Pale yellow/-	Blue fluorescent
	3	0.61	Pale yellow green/-	Blue fluorescent
	4	0.95	Green	Orange/Red fluorescent
Callus of week (6)	1	0.24	Pale yellow green/-	Blue fluorescent
	2	0.54	Pale yellow green/-	Blue fluorescent
	3	0.63	Pale yellow/-	Blue fluorescent
	4	0.94	Green	Orange/Red fluorescent
Callus of week (8)	1	0.26	Pale yellow green/-	Blue fluorescent
	2	0.56	Pale yellow green/-	Blue fluorescent
	3	0.61	Pale yellow/-	Blue fluorescent
	4	0.95	Green	Orange/Red fluorescent

*Values represent MEANS calculated from triplicates experiments. (-) means these sports are almost invisible in some.

Table 4. TLC remarks of alcoholic extract components of *S. hermonthica* developed on system III (Ethyl acetate/Formic acid/Water; 68:08:08).

Plant parts	No. of spots	R_f (values)*	Separated spots remark	Spots under UV-lamp at (365nm)
Shoot	1	0.74	Pale yellow/-	Blue Fluorescent
	2	0.88	Pale yellow/-	Blue Fluorescent
Haustoria	1	0.83	Pale yellow	Blue Fluorescent
Callus of week (4)	1	0.75	Pale yellow green/-	Blue Fluorescent
	2	0.88	Pale yellow/-	Blue Fluorescent
Callus of week (6)	1	0.74	Pale yellow/-	Blue Fluorescent
	2	0.88	Pale yellow/-	Blue Fluorescent
Callus of week (8)	1	0.74	Pale yellow/-	Blue Fluorescent
	2	0.86	Pale yellow/-	Blue Fluorescent

* Values represent means calculated from triplicates experiments. (-) means these sports are almost invisible in some.

documented (Valero and Salmeron, 2003). The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo et al., 1996; Iwu et al., 1999). The antimicrobial activities of the different extracts of the different parts of *S. hermonthica* were assayed *in vitro* using agar well diffusion method. Three bacterial strains, *S. aureus* ATCC 25923, *E. coli* ATCC25922, *Ps. aeruginosa* ATCC 27853, and one fungal species *C. albicans* ATCC7596 were used. The results represent the microbial growth inhibition by petroleum ether, ethanol and aqueous extracts of *S. hermonthica* at a concentration 5 mg/ml (Tables 6).

Table 5. TLC remarks of alcoholic extract components of *S. hermonthica* developed on system IV (Butanol/Acetic acid/Water; 100:25:125).

Plant parts	No. of spots	R_f (values)*	Separated spots remark	Spots under UV-lamp at (365nm)
Shoot	1	0.47	Pale yellow green/-	Blue Fluorescent
	2	0.90	Pale yellow green/-	Blue Fluorescent
	3	0.96	Pale green	Orange/red fluorescent
Haustoria	1	0.10	Pale yellow/-	Blue Fluorescent
	2	0.91	Pale yellow/-	Blue Fluorescent
Callus of week (4)	1	0.10	Pale yellow green/-	Blue Fluorescent
	2	0.55	Pale yellow/-	Blue Fluorescent
	3	0.93	Pale green	Orange/Red fluorescent
Callus of week (6)	1	0.12	Pale yellow green/-	Blue Fluorescent
	2	0.55	Pale yellow green/-	Blue Fluorescent
	3	0.91	Pale green	Orange/Red fluorescent
Callus of week (8)	1	0.10	Pale yellow green/-	Blue Fluorescent
	2	0.55	Pale yellow green/-	Blue Fluorescent
	3	0.91	Pale green	Orange/Red fluorescent

*Values represent Means calculated from triplicates experiments. (-) means these sports are almost invisible in some.

Table 6. Antimicrobial activities of *S. hermonthica* extracts agains tested standard microorganisms.

		Zones of inhibition (mm)*			
Plant parts	**Standard microorganisms**	**Ether extract**	**Ethanol extract**	**Water extract**	**Kanamycin antibiotic**
Upper parts (intact plant)	*S. aureus*	0.00	5.70	0.00	21.00
	P. aeruginosa	0.00	3.30	0.00	20.00
	E. coli	0.00	3.00	0.00	12.00
	C. albicans	1.00	2.70	0.00	n.t
Haustorium (intact plant)	*S. aureus*	0.00	2.70	0.00	21.00
	P. aeruginosa	0.00	3.70	1.30	20.00
	E. coli	0.00	2.00	0.00	12.00
	C. albicans	1.00	3.00	0.00	n.t
Callus	*S. aureus*	0.00	3.00	0.00	21.00
	P. aeruginosa	0.00	2.00	3.70	20.00
	E. coli	0.00	4.00	0.00	12.00
	C. albicans	1.00	2.00	0.00	n.t

*Values represent means of triplicates; n.t = not tested.

Controls were maintained where pure solvents were used instead of the extracts. Moreover, Kanamycin antibiotic at a concentration of 5 mg/L was used against bacterial strains as positive control (Figure 2). The petroleum ether extracts of different parts of *S. hermonthica* showed no antibacterial properties against all bacterial strains and weak antifungal activity against *C. albicans* with 1.00 mm inhibition zone. In different *Striga* spp. Hirematch et al. (1996), found that the petroleum ether extract of both *Striga densiflora* Benth and *Striga orobanchioiedes* Benth has an antibacterial activity against pathogenic and non-pathogenic bacteria. The inactivity of ether extract of *S. hermonthica* may be due to the absence or insufficient and effective concentration of the antimicrobial agents in the petroleum ether extract of *S. hermonthica*. On the other hand, the ethanol extracts

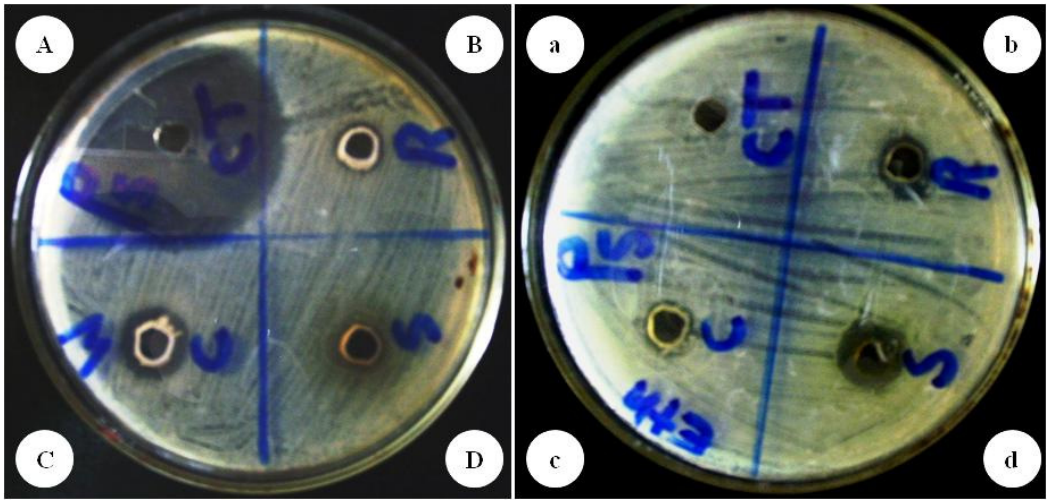

Figure 2. Antimicrobial activity of *S. hermonthica* extracts on *P. aeroginosa*, Left: the effects of water extracts (A) Kanamycin, (B) Intact plant (haustoria), (C) Callus and (D) Intact plant (shoot); Right: the effects of ethanol extracts (a) negative control, (b) Intact plant (haustoria), (c) callus and (d) Intact plant (shoot).

of almost all parts of *S. hermonthica* were used in this study (callus, upper-parts or under-ground parts) exhibited antimicrobial activity with various actions against the tested microorganisms. The maximum antimicrobial activity was achieved by ethanol extracts of the upper-parts of *S. hermonthica* against Gram-positive *S. aureus* ATCC25923 with inhibition zone of 5.7 mm as highest inhibition zone among all extracts, followed by ethanol extract of callus which showed activity against *E. coli* ATCC25922 with 4.00 mm inhibition zone. The lowest activity was obtained from ethanol extracts of both callus and under-ground parts with 2.00 mm zone inhibition against *C. albicans* ATCC7596 and *P. aeruginosa* ATCC27853 in callus and *E. coli* ATCC25922 in under-ground parts. Previous work done by Hirematch et al. (1997) on *Striga sulphurea* revealed that the ethanol (95%) extract exhibited high activity against *S. aureus*, *E. coli*, *P. aeruginsoa* and *Aspergillus niger*. These findings support our results obtained with the ethanol extracts, which may be due to the richness of the ethanol extract with active principle compounds, such as flavonoids, cardiac glycosides, alkaloids. The aqueous extracts of different parts of *S. hermonthica* showed no effect against all tested microorganisms, except the water extracts of callus and under-ground parts, which exhibited activity against *P. aeruginosa* ATCC27853. Kanamycin antibiotic was used at concentration 5 mg/ml against tested bacterial strains, the results obtained revealed high differences between Kanamycin and *S. hermonthica* extracts. The variations in the results obtained from different extracts of the different parts of *S. hermonthica* may reflect the influence of the tested microbial strains, plant parts and solvents used in the extraction, which play important roles as restriction factors affecting the antimicrobial activity. The differences in the antimicrobial effects of the different parts of *S. hermonthica* may strongly be attributed to the phytochemical properties and differences among the various parts of the plant. More comprehensive studies regarding the phytochemistry and the pharmaceutical properties of this plant will eventually enrich the knowledge about the scientific aspect of its medicinal uses.

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