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Phytochemical Screening, Antimicrobial, Antioxidant and Anticancer Activities of Yemeni *Croton socatranus*

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Abstract:

Croton socatranus is a shrub or small tree endemic to Socotra Island and is widespread on the island. It has some interesting ethnomedicinal uses. The dry powder was subjected to extraction till exhaustion using methanol. The methanol extract was evaluated phytochemically, chromatographically, and for its antimicrobial and antioxidant activity. Toxicological and cytotoxic effects were also investigated. Microscopic examination of *C. socatranus* aerial parts confirmed the presence of xylem vessels, sclerenchymatous cells, slender-shaped fibers, vessel elements with scalariform thickenings, palisade cells, paracytic stomata, and stellate trichomes. Phytochemical investigation showed that the methanol extract possessed alkaloids, high levels of phenolic compounds, tannins, flavonoids, triterpenes, diterpenes, phytosterol, and saponin. Notably, the methanol extract of *C. socatranus* did not cause any mortality or behavioral changes in the rats even at the highest tested dose (5000mg/kg of methanol extract). It showed inhibition zone against *Streptococcus pneumonia* and *Escherichia coli* and MIC values were between 156.25- 655 µg/ml for gram-positive strains while 625-2500 µg/ml for gram-negative bacteria. Antifungal activity was recorded against ten tested fungal species. Furthermore, a dose-dependent antioxidant activity was observed. *C. socatranus* extract displayed moderate cytotoxic activity against eight cancerous cell lines. The extract was highly effective on MCF-7, HepG-2, and RD cell lines when compared with other cell lines and the IC50 was less than 30 µg/ml which suggests the presence of selective cytotoxic compounds. Further investigations are required to understand the possible mechanism(s) of action of this extract on various cancer cells and the isolation of active phytochemicals.

INTRODUCTION

Socotra (Yemen) is located in the Indian Ocean (Gulf of Aden). The area is about 3549 sq. km and the maximum altitude is 1506 m (Tardelli and Baldini, 2000). The vegetation of Socotra hosts an unprecedented heritage of endemic species that have originated from radiation processes within the archipelago, or phyletic evolution from Paleo-African, African-mesic, and mesic-tropical Asian taxa (De Sanctis *et al.*, 2013). The Euphorbiaceae family is additionally referred to as the family Euphorbiaceae which is one of all the foremost diversified families of flowering plants which is taken into account because of the sixth largest family. There are about 300 genera and over 8000 species constituting the rosid dicot family. In India, 73 genera and 410 species are reported (Divya *et al.*, 2011). In Socotra Island, Yemen, there are 3 endemic croton species: *Croton sulci Fructus, C. socatranus,* and *C. sarcocarps* (Miller and Morris, 2004). Croton plants are used widely and variedly in folk medicine everywhere the globe.

The croton genus occurs mostly in tropical regions worldwide, but also has some representatives in subtropical and northern temperate areas, and its main centers of diversity in the Neotropics are Brazil, the West Indies, and Mexico (Caruzo, 2013).

Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumors and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis, which is the major cause of death from cancer. There were around 12 million new cancer cases and 7 million cancer deaths worldwide in 2008, and 13-17 million deaths projected for 2030 (Mulcahy, 2008).

This work demonstrated the antimicrobial activity of *C. socatranus* aerial parts methanol extract on some bacterial and fungal strains, similar researches have to be carried out on pathogenic microbes other than those used in the present study. Also, the possible mechanism of actions of both the antibacterial and antifungal activities must be studied.

In addition, aims to explore the antitumor potential of methanol extract of *C. socatranus* aerial parts against several human cancer cell lines (HepG2, HCT-116, MCF-7, A549, PC-3, HEp-2, and HeLa) and their screening for phytochemicals present.

MATERIALS AND METHODS

Croton socatranus aerial parts (stem, leaves, and flowers) were manually collected in September 2019 from Socotra Island. The plant was identified at the office of Environment Protection Authority, Socotra branch. A specimen (voucher no. 424) was deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University, Yemen.

Extraction and Fractionation

Fresh plant parts (3.75 kg) were cleaned and air-dried under shade for 4 weeks, then ground into a fine powder employing a grinder. 1.523 kg of the powder was macerated in methanol for about one week. This procedure was repeated until the powder was exhausted. The filtrate was concentrated employing a rotary evaporator at 45- 50 °C. The ultimate word yield of extract was then calculated by subtracting the extract from the first weight of the powder. Subsequently, the extract was stored in airtight containers at temperature until use. About 181gm of methanol extract was dissolved in 2000ml of methanol and water so partitioned with n-hexane, chloroform, ester, and butanol respectively. Each fraction was dried over anhydrous sulfate and evaporated to dryness to yield.

Pharmacogenetic Assessment Macroscopic and Microscopic Examination

The color, shape, size, taste, and odor of the plant powder were determined. The microscopic study was carried out by preparing a thin film of powder, chloral hydrate was added and then evaporated until drying after those 2 drops of

glycerin were added to prevent the slide from drying. With the help of a light microscope (Cambridge Instruments, USA), different key elements

Qualitative Phytochemical Tests

Chemical tests were performed for identification of alkaloids, carbohydrates, fixed oils, glycosides, flavonoids, anthraquinones, phenolic compounds, tannins, phytosterols, proteins, saponins, carotenoids, and mucilage from methanol extract of *C. socatranus* (C.S.T) following (Banu and Cathrine, 2015).

Quantitative Phytochemical Determination

This test was done at Shiba Pharma Company, Sana'a, Yemen to estimate the relative amount of carbohydrates, alkaloids (Smibert *et al.*, 1994), total phenolic compounds, flavonoids, (Zhishen *et al.*, 1999), and sterols (Sabir *et al.*, 2003).

Quantitative determination of major minerals

Quantitative determination of major minerals of the methanol extract of *C. socatranus* was done at Shiba Pharma Company, Sana'a, Yemen using spectrophotometric method (calcium, phosphorus, magnesium, and chloride) and flame photometer (sodium and potassium).

Biological activities of *Croton socatranus* Experimental Animals

All animals were fed with standard animal feed and water impromptu. The animals were acclimatized to the laboratory conditions for five days before experimentation. All experiments distributed were approved by the Institutional Ethical Committee, Faculty of medication and Health, Sana'a University (334-12/03/2019), and were conducted keeping with the quality guideline for the employment of laboratory animals (National Institutes of Health, 1986; Sabir *et al.*, 2003).

Acute Oral Toxicity

Acute oral toxicity was determined keeping with the Organization for Economic Cooperation and Development (OECD) guidelines. In this experiment, 30 rats (123 gm -178.8 gm body weight) divided into five groups each of 6 animals received oral gradually increased doses from 0.5 to 5g/kg (control group, 0.5gm/kg, 1gm/kg, 2.5gm/kg, and 5gm/kg) of the methanol extract dissolved in water. While the control group received the water at the identical volume. After administration at the third hour on a primary day, the rats were administered throughout the subsequent 48 hours daily thereafter for 14 days (Newman and Cragg, 2014). At the end of the experiment, the animals underwent euthanasia with a high dose of thiopental (100 mg/kg IP) (El Gamal, 2010).

Antioxidant Activity

This assay was done at Shiba Pharma Company, Sana'a, Yemen. Evaluation of antioxidant activity was done by the DPPH atom scavenging assay (Yen and Duh, 1994). Absorbance measurements were recorded after 30 minutes incubation within the dark with a UV-visible spectrophotometer. The decrease in absorbance at 515 nm makes up my mind continuously. The absorbance of the DPPH radical without antioxidant (control) and thus the reference compound vitamin was also measured. The share inhibition (PI) of the DPPH radical was calculated per the formula; PI = Where AC = Absorbance of the control (blank, without extract) and AT= absorbance of the sample +DPPH (Subhasree *et al.*, 2009).

Antibacterial Susceptibility Assays Well Diffusion Assay

The antibacterial activity was investigated on methanol extract by the agar diffusion method using Mueller-Hinton agar medium (Abdelrahman *et al.*, 2017). Four-gram negative stains were used (*Escherichia coli, Proteus mirabilia, Klebsiella pneumonia, and Pseudomonas aeruginosa*).

Also, four gram-positive strains were used in studying the antibacterial activity (*Staphylococcus aureus, Streptococcus pneumonia, Streptococcus pyogenes, and Methicillin-resistant S. aureus MRSA*) (Iqbal *et al.*, 2015).

Bacterial Minimum Inhibitory Concentration

Bacterial species that were inhibited by using the methanol extract of *C. socatranus* were used to determine MIC by the dilution method using the agar diffusion method (Abdelrahman *et al.*, 2017).

Antifungal Susceptibility Assays

The antifungal activity was investigated by the agar diffusion method using Sabouraud's agar medium (Kalim *et al.*, 2016) at the laboratories of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Fungal Minimum Inhibitory Concentrations

The antifungal activities were tested in terms of minimum inhibitory concentration (MIC) using the agar diffusion method (Kumar and Jha, 2017).

Antitumor Activity of the Methanol Extract - MTT assay

The tetrazolium-based colorimetric assay (MTT) was accustomed to determine the cytotoxicity of *C. socatranus* methanol extract mathematic on 11 cell lines which are MCF-7, HCT-116, HepG-2, PC-3, A-549, HELA, HEP-2, M-NFS-60, RD, CHO-K1, and CACO-2 cell lines. A non-cancerous human lung fibroblast cell line (MRC-5) was used as a customary control cell line for comparison. The assay relies on the flexibleness of mitochondrial dehydrogenase enzyme present in viable cells to cleave the tetrazolium rings of the yellowness MTT dye and from dark purple formazan crystals which are largely impermeable to cell membranes, finally lands up in its accumulation within the cells which provides a quantitative determination of viable cells (de Sá-Haiad *et al.*, 2009). Cancer cells were plated onto 96 well plates at a cell density of 2×105 cells/ml per well in 100 µl of Roswell Park Memorial Institute medium (RPMI 1640) and allowed to grow in an exceedingly CO2 incubator for twenty-four h (37 ˚C, 5 % CO_2). The medium was then removed and replaced by a fresh medium containing different concentrations of the sample (12.5, 25, 50, 100, 200, and 400µg/ml) and incubated for 48 hours (37 ˚C, 5 % CO_2). At the very best of the treatment period, the mixture of media and sample was removed and cells were incubated with 200 µl of fifty MTT solution/well to permit to metabolization of the dye into a colored-insoluble formazan complex for five hr. After that, the MTT was removed and 200 µl DMSO was added to every well to dissolve the MTT metabolic product. Then the plate was shaken at 150 rpm for five min and also the absorbance of the samples was measured employing a microplate reader at wavelength 570nm. Untreated cells are used as an impression of viability (100 %) and also the results are expressed like viability relative to the control. the kids of viability were calculated using the subsequent formula; % viability = – – × 100 Where AT = Absorbance of treated cells (drug) AB = Absorbance of blank (only media) AC = Absorbance of control (untreated cell lines) the common cell viability percentage obtained from triplicate determinations at each concentration was plotted as a dose-response curve using Graph Pad Prism version 5.0 and also the inhibition concentration at 50% (IC50) values (concentration of extracts or standard drug inducing 50% inhibition of cancer cells) determined from the dose-response curve by the nonlinear statistical procedure.

Doxorubicin Hydrochloride was used as standard and tested at identical concentrations of the methanol extract.

Statistical Analysis

All data were analyzed by using SPSS Software Version 26. All results were tired triplicate and were expressed because of the mean ± SD from three different experiments. Independent (student) t-test was used to measure statistical differences between the mean values. The statistical difference was indicated with value $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS AND DISCUSSION

This study aimed to identify *C. socatranus* aerial parts and to evaluate its different biological activities. Microscopic examination of aerial parts powder of *C. socatranus* demonstrated the presence of xylem vessels, sclerenchymatous cells, slender-shaped fibers, vessel elements with scalariform thickenings, palisade cells, paracytic stomata, and stellate trichomes (Figure 1). Paracytic stomata are one of the characteristics of the Croton genus (de Sá-Haiad *et al.*, 2009). This type of stomata was abundant in *C. socatranus* powder. In the Euphorbiaceae, particularly the genus croton, stellate trichomes are present (Yang *et al.*, 2010).

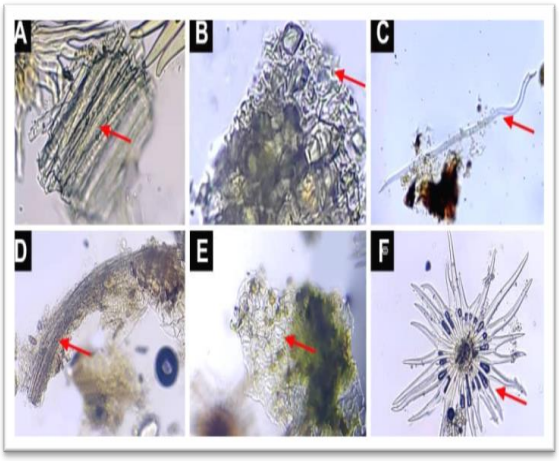

Fig. 1. Major Key elements of *C. socatranus* aerial parts powder

A) xylem vessels**. B)** sclerenchymatous cells. **C)** slender-shaped fibers. **D)** vessel elements with scalariform thickenings. **E)** palisade cells, paracytic stomata. **F)** stellate trichomes.

The phytochemical investigation of methanol extract of *C. socatranus* aerial parts showed the presence of alkaloids, phenolic compounds, tannins, flavonoids, triterpenes, diterpenes, phytosterol, and saponin (Table 2). These phytochemical results agreed with the results of some croton species like *C. reuse* and *C. variegatum* (Dhole *et al.*, 2012).

Table 1. Qualitative phytochemical analysis of *C. socatranus* methanol extract.

Sr.No.		Test	Result
1	a	Mayer's test	+ve
	b	Wagner's test	+ve
	c	Hager's test	+ve
2	a	Molisch's test	+ve
	b	Fehling's test	+ve
	c	Benedict's test	+ve
3	a	NaOH (Alkaline test)	+ve
	b	Lead acetate	+ve
	c	Shinoda test	+ve
4		Foam test	+ve
5	a	Xanthoprotein test	-ve
	b	Ninhydrin's test	-ve
6	a	Lieberman Burchard's test	+ve
	b	Salkowski's test	+ve
7		Copper acetate	+ve
8	a	Ferric chloride test	+ve
	b	Gelatin test	+ve
9		Ferric chloride test	+ve
10		1% aqueous hydrochloride	+ve
11	a	Modified Borntrager's test	-ve
	b	Cardiac glycosides	+ve
12	a	Free anthraquinone	-ve
	b	Combined anthraquinone	-ve
13	a	Spot test	+ve
	b	Saponification test	+ve
14		85% sulphuric acid	-ve

Quantitative determination of the phytoconstituents was carried out for the dry methanol extract of *C. socatranus* by various standard methods (Figures 2, 3, 4, 5, and 6). The highest concentration of the evaluated secondary metabolites was for tannins (62.2 mg/gm tannic acid equivalent) followed by total phenolics (51.6mg/gm gallic acid equivalent) and flavonoids (36.9mg/gm rutin equivalent). The concentration of alkaloids and sterols was (0.167 mg/gm atropine equivalent) and (0.1mg/gm cholesterol equivalent) respectively (Table 2). These results are generally higher than the results obtained by a previous study which was done on *C. bonplandianus* stem powder, the tannins content was 51.94±0.38 mg/100g gallic acid equivalent, phenolic content was 67.37±0.46 mg/g gallic acid equivalent and total flavonoid content was 3.86±0.12 mg/g (Dutta *et al.*, 2014).

Table 2. Alkaloids, total phenolic compounds, tannins, flavonoids, and sterols contents of *C. socatranus* methanol extract.

Components	Content (mg/gm)
Alkaloids	0.167 mg/gm atropine equivalent
Total phenolic compounds	51.6 mg/gm gallic acid equivalent
Flavonoids	36.9 mg/gm rutin equivalent
Tannins	62.2 mg/gm tannic acid equivalent
Sterols	0.1mg/gm cholesterol equivalent

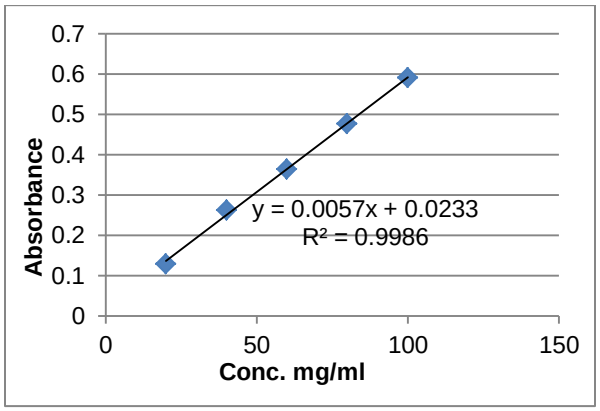

Fig. 2. Calibration curve of glucose for carbohydrate content determination.

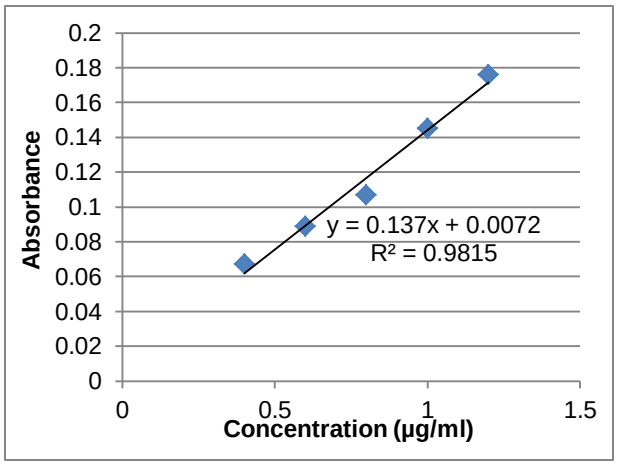

Fig. 3. Calibration curve of atropine for alkaloids content determination.

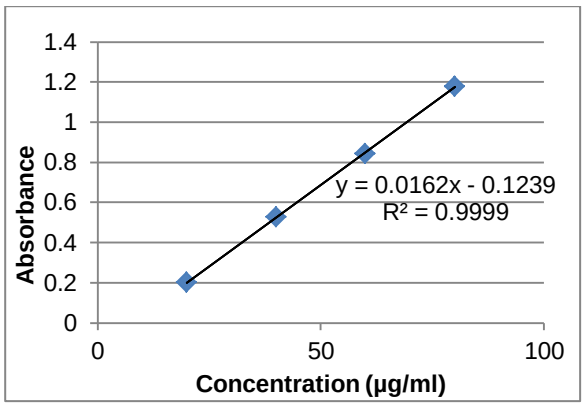

Fig. 4. Calibration curve of gallic acid for total phenolic compounds content determination.

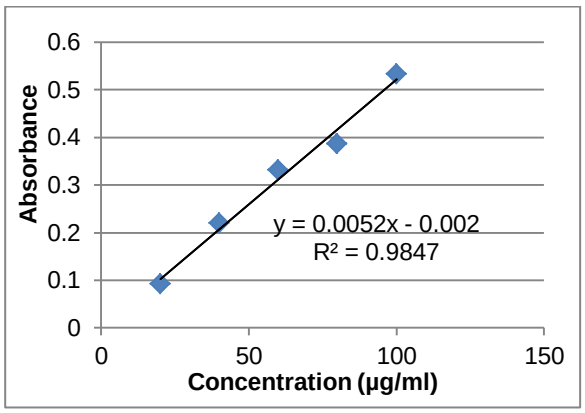

Fig. 5. Calibration curve of rutin for flavonoids content determination.

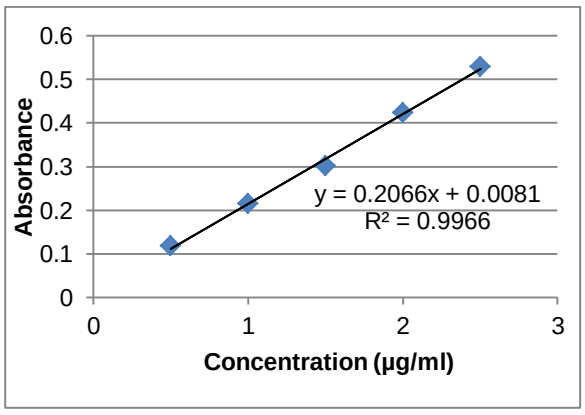

Fig. 6. Calibration curve of cholesterol for sterols content determination.

The evaluation of the toxicity of plant extracts is indispensable to consider the treatment is safe; it enables the definition of the intrinsic toxicity of the plant and the effects of acute overdose (Seoudi *et al.*, 2009).

The methanol extract did not show any mortality or toxicological or behavioral changes in the rats and mice even at the highest dose used (5000mg/kg of methanol extract). These results are agreed with the results of (Mbiantcha *et al.*, 2013) studies on the assessment of toxicity of *C. macrostachyus* stem bark although higher doses of *Croton macrostachyus* were used (12- 16 g/kg b.wt.). In another acute toxicity study of leaf methanol extract of *C. macrostachyus* in mice, a single oral dose of 2 and 5 g/kg did not cause any mortality within the first 24 h and up to 14 days observation period which suggested the safety profile of *C. macrostachyus* methanol extract in the study mice (Bantie *et al.*, 2014).

The determined lethal dose (LD50) of methanol extract of *C. socatranus* aerial parts was >5000g/kg. The LD50 of *C. zambesicus* methanol extract was found to be above the dose of 12 g/kg (Onwusonye *et al.*, 2016). According to (Hodge and Sterner, 2005), any compound with oral LD50 of 5000mg/kg or more in rats should be considered practically harmless. Meaning that no substantial toxic effect occurred in animals that were administered *C. socatranus* methanol extract. These results suggest that the margin of safety of the methanol extract is high.

The methanol extract of *C. socatranus* showed antibacterial activity against gram-positive bacterial strains (Table 3). The MIC values of the methanol extract against gram-positive strains were between 156.25- 655 µg/ml (Table 4). Antibacterial activity was also observed against gram-negative bacterial strains (Table 5). The MIC of this extract against gram-negative bacteria was between 625-2500 µg/ml (Table 6).

Antibacterial activity has been reported from many *Croton* genera. A study found MICs between 15-250 mg/ml after testing methanol extract of *C. macrostachyus* against four bacterial strains (*E.coli, P.aeruginosa, M.lutea,* and *B. cereus)* (Wagate *et al.*, 2010). Benzylpenicillin with MIC of 0.6mg/ml and streptomycin with MIC of 0.25mg/ml was used for gram-positive and gram-negative bacteria, respectively.

Results from (Selowa *et al.*, 2010), showed that methanol extracts from the three *Croton* species: *C. megalobotrys, C. steenkapiamus,* and *C. sylvatic* produced different results. *C. megalobotrys* inhibited only *E. coli* and at higher concentrations, *C. steenkapiamus* inhibited *P. aeruginous*, but was inactive against *E. coli*, *S. aureus,* and *E. faecalis*. In comparison, *C. sylvatic* inhibited weakly all the test organisms at the constant concentration of 1.25 mg/ml.

Table 3. Antibacterial activity of *C. socatranus* (methanol extract) against gram-positive bacteria.

Bacterial strain	Antibacterial activity (inhibition zones in mm)					
	1mg/ml		10mg/ml		50mg/ml	
	C.S.T	Std	C.S.T	Std	C.S.T	Std
Streptococcus pneumonia	10.33±0.58*	30.33±0.58	12.33±0.58*	35.00±0.00	15.33±0.58*	40.33±0.58
Streptococcus pyogenes	10.33±0.58*	28.33±0.58	13.33±0.58*	35.33±0.58	15.33±0.58*	42.33±0.58
Staphylococcus aureus	12.33±0.58*	28.33±0.58	14.33±0.58*	33.33±0.58	16.33±0.58*	39.33±0.58
Methicillin-resistant *S. aureus* (MRSA)	8.67±0.58*	24.67±0.58	12.33±0.58*	35.00±0.00	15.33±0.58*	40.67±0.58
The test was done using the diffusion agar technique, Well diameter = 5.0 mm, Test volume = 100 µl, Std = Gentamycin, C.S.T= methanol extract of *C. socatranus*. *p-vale is < 0.05						

Table 4. Minimum inhibitory concentration (MIC) of C.S.T against Gram +ve bacteria.

Tested microorganisms	MIC (µg/ml)	
	C.S.T	Standard
S. aureus	156.25	1
S. pneumonia	625	1-4
S. pyogenes	625	1

*C.S.T: Methanol extract of *C. socatranus*, Standard= Gentamycin.

Table 5. Antibacterial activity of *C. socatranus* (methanol extract) against gram-negative bacteria.

Bacterial strain	Antibacterial activity (inhibition zones in mm)					
	1mg/ml		10mg/ml		50mg/ml	
	C.S.T	Std	C.S.T	Std	C.S.T	Std
Escherichia coli	9.33±0.58*	24.33±0.58	10.33±0.58*	29.67±0.58	14.33±0.58*	33.67±0.58
Pseudomonas aeruginosa	NA	23.00±0.00	11.67±0.58*	27.67±0.58	14.33±0.58*	30.33±0.58
Proteus mirabilis	12.00±0.00*	30.33±0.58	15.33±0.58*	35.00±1.00	16.33±0.58*	37.00±5.20
Klebsiella pneumonia	8.33±0.58*	24.67±0.58	8.67±0.58*	30.00±0.00	10.00±0.00*	32.67±0.58

The test was done using the diffusion agar technique, Well diameter = 5.0 mm and (100 µl was tested), NA=No activity, Std= Gentamycin, C.S.T= methanol extract of *C. socatranus*. *p-value is < 0.05

Table 6. Minimum inhibitory concentrations values of C.S.T against gram –ve strains in µg/ml

Tested microorganisms	MIC (µg/ml)	
	C.S.T	**Standard**
E. coli	1000	4.9
P. mirabilis	1000	4.9
K. pneumonia	625	4.9
P. aeruginosa	2500	9.8

C.S.T: Methanol extract of *C. socatranus*, Standard= Gentamycin.

Antifungal activity was recorded against ten tested fungal species (Table 7) and the MIC value of the methanol extract against fungal strains was between 0- 2500 µg/ml (Table 8). The antifungal activity is shown by many croton species. For example, the antifungal investigation of *C. tiglium* indicates that; methanol extract showed significant antifungal activity against two fungal strains (*A. niger* and *R. Oryza*) as compared to some standards like ketoconazole, econazole, nystatin, amphotericin, clotrimazole, and miconazole (Zahid and Mughal, 2019). These activities (antibacterial and antifungal) could be due to the high concentration of tannins in the methanol extract (Hussain *et al.*, 2016; Kalim *et al.*, 2016; Nigussie *et al.*, 2021; Suurbaar *et al.*, 2017).

The present study revealed that the methanol extract showed a dose-dependent antioxidant activity (Table 9) and IC 50 value was recorded as 102 µg/ml (Table 10). DPPH technique is a widely used method to evaluate the free radical scavenging and antioxidant activities of various plant extracts (Lee *et al.*, 2003).

The antioxidant activity of the methanol extract generally is due to the presence of a good concentration of total phenolic compounds like flavonoids that possess good antioxidant activities (Van Acker *et al.*, 1996). Previous research has revealed the strong relationship between the total phenolic content of plants and their antioxidant potential (Klimczak *et al.*, 2007). Other studies of the Croton genus are also proved the strong antioxidant activities which are greatly associated with the presence of phenolic compounds (Nath *et al.*, 2013). The results of the antioxidant activity of our research are confirming such findings.

Table 7. Antifungal activity of the methanol extract of *C. socatranus* against fungal strains.

Test microorganisms	Antibacterial activity (inhibition zones in mm)	
	C.S.T	Standard
Aspergillus fumigatus	17.00±1.73*	25.7 ± 0.6
Syncephalastrum racemosum	19.33±1.15*	23.3±0.6
Geotricum candidum	19.67±1.53*	24.7±0.6
Candida albicans	NA	22.0±1.0
Aspergillus niger	21.33±1.15	23.3±1.2
Cryptococcus neoformans	14.33±1.53*	22.3±1.5
Candida tropicalis	NA	21.7±1.5
Penicillium expansum	17.33±0.58*	20.7±1.5
Microsporum canis	18.33±0.58*	19.7±0.6
Trichophyton mentagrophytes	14.33±0.58*	20.0±1.0

The test was done using the diffusion agar technique, well diameter: 6.0 mm …… …… (100 µl was tested),
NA: No activity, data are expressed in the form of mean ± SD.
C.S.T: Methanol extract of *C. socatranus* tested at 10 mg/ml, Standard= Amphotericin B was tested at 1 mg/ml
* P-value is < 0.05

Table 8. Minimum inhibitory concentrations values of C.S.T against fungal strains in µg/ml

Tested microorganisms	MIC (µg/ml)	
	C.S.T	**Standard**
A. fumigatus	1250	2.4
S. racemosum	1250	4.9
G. candidum	625	2.4
C. albicans	NA	4.9
A. niger	625	2.4
C. neoformans	2500	2.4
C. tropicalis	NA	4.9
P. expansum	625	4.9
M. Canis	1250	19.53
T. mentagrophytes	2500	19.53

NA: No activity, C.S.T: Methanol extract of *C. socatranus*, Standard= Amphotericin B.

Table 9. DPPH % of inhibition by *C. socatranus* versus ascorbic acid.

Sample concentration (µg/ml)	Std (Ascorbic Acid) *	C.S.T
160	96.76±0.02	75.23±0.03 a
80	96.35±0.01	40.87±0.12 a
40	96.35±0.01	22.81±0.04 a
20	96.47±0.10	13.00±0.02 a
10	96.00±0.01	7.39±0.02 a
5	71.78±0.04	5.52±0.01 a

Reference drug. a p-value <0.001 compared to the reference drug. C.S.T. (methanol extract), and Std (ascorbic acid standard).

Table 10. IC50 values of *C. socatranus* against DPPH.

Fraction	IC 50 (µg/ml)
C.S.T	102

The anticancer effect of *C. socatranus* methanol extract on selected cancer cell lines was assessed by using the MTT assay method. It displayed a variable cytotoxic activity against all eleven cancerous cell lines expressed by different IC50 values (Table 11). It showed high efficacy on HepG-2, MCF-7, and RD cell lines (IC50<25µg/ml). Our cytotoxic assay results are agreed with the anticancer activity of results of the methanol extract of the leaves/twigs, roots,

and stem bark of *C. argyratus*. These extracts displayed toxicity to human lung *cancer* cell lines with an IC50 value of <5.0 µg/ml. Also, the methanol extract of the root of Croton membranous exhibited markedly cytotoxic activities against the DLD-1 (colon adenocarcinoma) and MCF-7 (breast cancer) cells with IC50 = 16.0 and 17.4µg/ml respectively (Nath *et al.*, 2013).

Table 11. Cytotoxic activity (IC50) of *C. socatranus* methanol extract against various cancer cell lines.

	Cytotoxic activity expressed as IC_{50} (µg/ml)										
Cell line	MCF-7	HCT-116	HepG-2	PC-3	A-549	HELA	HEP- 2	M-NFS-60	RD	CHO-K1	CACO-2
C. socatranus	22.5 ± 1.54	37.2 ± 2.27	21 ± 1.44	41.7 ± 0.40	39.7 ± 2.92	38.9 ± 5.46	46.1 ± 2.32	98.7 ± 11.3	23 ± 1.46	36.6 ± 5.77	32.2 ± 1.61
Doxorubicin	0.62 ± 0.08	0.55 ± 0.04	0.67 ± 0.02	1.43 ± 0.57	1.36 ± 0.12	2.05 ± 0.29	3.47 ± 1.23	1.87 ± 0.14	3.9 ± 0.43	0.17 ± 0.02	1.93 ± 0.24

MCF-7 (breast cancer), HCT-116 (colon cancer), HepG2 (hepatocellular carcinoma), PC-3 (prostate cancer), A-549 (lung adenocarcinoma), HeLa (cervical cancer), HEP- 2 (Epidermoid carcinoma), M-NFS-60 (Myelogenous leukemia), RD (Rhabdomyosarcoma), CHO-K1 (Chinese hamster ovary cancer) and CACO (colorectal adenocarcinoma).

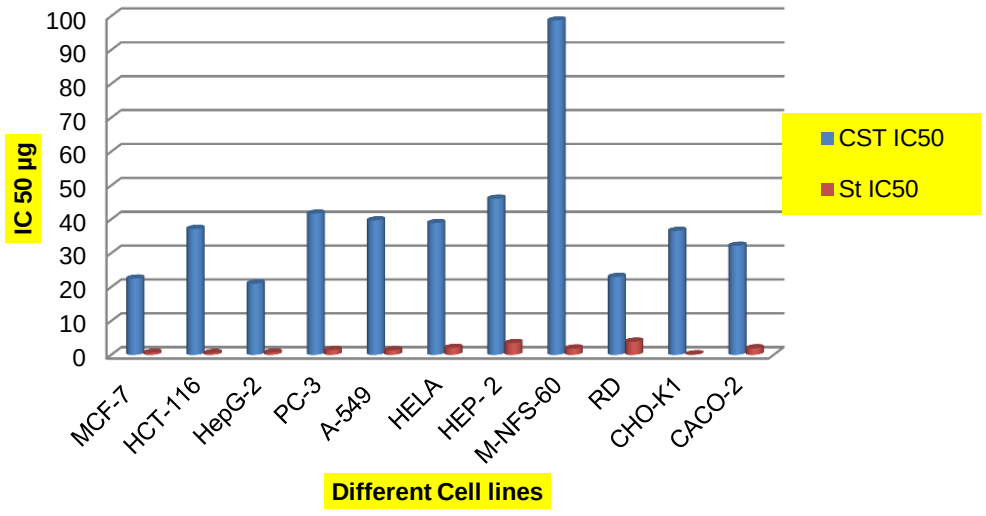

Fig. 7. Cytotoxic activity of *C. socatranus* methanol extract to different cancer cell lines versus doxorubicin (C.S.T = methanol extract of *C. socatranus*.

The American Cancer Institute sets the criteria of 30µg/ml as the upper IC50 limit considered promising for the purification of a crude extract (Suffness, 1990). Finally, it is important to mention that, this study is the first study that was done to fully investigate *the C. socatranus* plant pharmacognostically, phytochemically, toxicologically, and pharmacologically (antimicrobial, antioxidant, and anticancer activities).

CONCLUSION

In conclusion, the study provides information on the phytochemical constituents, antimicrobial, antioxidant, and anticancer effects of *C. socatranus* aerial parts. From this study, the presence of alkaloids, phytosterol, phenolic compounds, tannins, flavonoids, diterpenes, fixed oil, saponins, and tannins, were detected in the methanol extract did not show any sign of acute toxicity up to a dose of 5000mg/kg. It exhibited a dose-dependent antioxidant activity. Methanol extract of *C. socatranus* showed stronger antifungal activity against the tested fungal strains and various levels of anticancer activity on eleven cancer cell lines in a concentration-dependent manner. This extract was highly effective on MCF-7, HepG-2, and RD cell lines when compared with other cell lines and the IC50 was less than 30 µg/ml which suggests the presence of selective cytotoxic compounds. Further investigations are required to understand the possible mechanism(s) of action of this extract on various cancer cells and the isolation of active phytochemicals.

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CONFLICT OF INTEREST

The authors declare that this article's content has no conflict of interest.

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