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Research Article
Effects of Extracts from Five Plants on Some Biochemical Changes in Tomato Seedlings

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Abstract

Background and Objectives: Tomato is worldwide one of the most consumed fruit due to its nutritional and potentially health promoting properties. However, these properties can be influenced by many factors including bioregulators, biofertilizers and biopesticides. Since extracts/fractions from leaves of *Polyscias fulva* (*P. fulva*), *Cupressus lusitanica* (*C. lusitanica*), *Tephrosia vogelii* (*T. vogelii*), *Senna spectabilis* (*S. spectabilis*) and *Callistemon viminalis* (*C. viminalis*) are well known for their effects on plant grown and development, the present study was carried out to verify whether they could influence tomato seeds germination and seedlings growth through biochemical changes. **Materials and Methods:** Aqueous and methanol extracts were obtained by macerating the leaf powder into water and methanol, respectively. Methanol extracts were then partitioned into hexane, ethyl acetate and residual fractions. About 10 tomato seeds were treated with these extracts and fractions sprouted in 90 mm diameter petri dishes. After 15 days of experiment, the seedlings were harvested and some of their biochemical parameters were studied. **Results:** Treatments of tomato seeds with extracts and fractions resulted globally in an increase in total phenol and protein contents and in radical scavenging activity of seedling extracts. This was particularly true with *P. fulva*, *T. vogelii* and *S. spectabilis* extracts. Methanol extracts from all the five plant species, whatever the concentration, significantly ($p<0.001$) stimulated phenol synthesis in tomato seedlings. Furthermore, there was significant ($p<0.05$) increases in radical scavenging activities of tomato seedling extracts (RSa_{50} varying from 4.57-8.53) after treatments of tomato seeds with methanol extracts. The fractionation of the methanol extract better concentrated the antiradical activity in hexane fraction as compared to ethyl acetate and in some extent to residual fractions. Except for methanol extract, best stimulatory effect on protein/phenol synthesis and radical scavenging activities in tomato seedlings was observed at low concentration (0.156 mg mL^{-1}). **Conclusion:** These results showed that the tested plant extracts and fractions can influence some biochemical parameters in tomato seedlings. The results obtained in this work can be useful for tomato crop protection and production.

Key words: Tomato, seedlings, proteins, phenols, scavenging, allelopathy

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato is one of the most consumed fruits in the world. It reduced risk of cardiovascular diseases, digestive tract tumors, inflammatory processes, cardiovascular diseases, hypertension, diabetes and obesity[1]. It possesses antioxidant properties due to the presence of some secondary metabolites such as lycopene, flavonoids and carotenoids[2]. However, the synthesis of these useful substances in the plant can be influenced by many factors, including allelochemicals. Allelopathic interactions are known to be mediated by secondary metabolites released from a donor plant to the environment and can influence the growth and development of the target plant species in the ecosystems[3]. These interactions are known to be beneficial or detrimental for the target plant[4]. They can affect the seed germination rate and growth of seedlings by interfering with some important parameters including membrane permeability, oxidative and antioxidant systems, growth regulation systems, protein/enzyme synthesis and general metabolism[5,6].

Concerning detrimental aspect of allelopathy, Cruz-Ortega *et al.*[7] showed that oxidative stress is one of the mechanisms by which allelopathic plants become phytotoxic for others. To resist oxidative stress induced by allelochemicals, the target plant produces reactive oxygen species (ROS) in its vicinity[4] and modifies the activity of antioxidant enzymes such as superoxide dismutase, peroxidase[7] and ascorbic acid peroxidase[8]. The allelochemical compounds thus cause an imbalance in the antioxidant system[9] and then induce a negative effect on target plants. It is suggested that allelopathic phenomenon is partly due to impairment of DNA, RNA and protein biosynthesis[9].

In a previous study, it was showed that aqueous extracts of *Tephrosia vogelii*, *Cupressus lusitanica* and *Callistemon viminalis* exerted inhibitory effects on tomato seed germination, while the methanol extract, the hexane and ethyl acetate fractions of these three plants and that of *Senna spectabilis* and *Polyscias fulva* stimulated the germination of these seeds[10]. Their aqueous extracts increased the stem length while they reduced the root length. It was then hypothesized in this study that the observed effects, whether beneficial or deleterious, resulted either from an imbalance production of ROS and antioxidant substances or from reduction of protein synthesis or from both phenomena. The present study was therefore aimed at evaluating the influence of these extracts/fractions from the 5 plants on the antiradical properties, the total phenols and proteins contents in the tomato seedlings resulting from germination of treated tomato seed.

MATERIALS AND METHODS

The experiment was a factorial study with 5 plants, 5 extracts and 3 concentrations. For each plant species tested, 5 extracts and fractions were tested and each fraction was tested at 3 concentrations.

Plant materials

Plant materials consisted of leaves from the following species: *Callistemon viminalis*, *Tephrosia vogelii*, *Senna spectabilis*, *Cupressus lusitanica*, collected in January, 2014 at the campus of the University of Dschang as well as *Polyscias fulva* collected in May, 2014 in Dschang and Bafou in the Western administrative region of Cameroon. The plant materials were identified at the Cameroon National Herbarium in Yaoundé, where a voucher specimen was kept respectively, under the reference numbers 49872/HNC, 43546/HNC, 45740/HNC, 35436/HNC and 47801/HNC. The leaves of each plant species were dried in the shade for 3 weeks at 22±2°C. They were finely crushed in a mechanical mill and the resulting powders were used to prepare plant extracts.

Tomato seeds (Rio Grande, lot No 58480, packaging date September, 2013. Vikima Seed A/S from Denmark) respecting the EC Standard Norms, were purchased from Holland Farming Sarl, Cameroon. All experiments were conducted in a randomized complete block design. Treatments in each experiment were replicated three times and all experiments were done in triplicate. The initial seed germination count was carried out 2 days after sowing. The biochemical parameters were determined in seedlings on the 15th day of the experiment.

Chemical reagents and solvents: 2,2-Diphenyl-1-picryl-hydrazyl (DPPH•), naphthalene acetic acid were of technical grade from Sigma-Aldrich, Germany. Hexane, ethyl acetate and methanol (for extract preparation), gallic acid, folin-ciocalteu reagent, boric acid, sulfuric acid, bromocresol green and methyl red were of analytical grade from Sigma-Aldrich, Germany. Methanol used in antiradical scavenging activity was HPLC-grade, purchased from Fischer Scientific, France.

Preparation of plant extracts and fractions: Aqueous extracts were prepared by macerating, respectively, 2.5, 1.25 and 0.625 g of plant powder in 100 mL of distilled water for 24 h. These extracts were then filtered using Whatman paper No.1. Methanolic extracts were prepared by macerating each

plant powder (1,000 g) in 6 L methanol for 2 days and then filtered using Whatman paper No. 1. The evaporation of solvent was carried out using a rotary evaporator (Buchi R-200) under vacuum at 40°C. The extracts obtained were placed in an oven at 40°C for 24 h to remove residual solvent. The methanol extracts were successively and separately partitioned using n-hexane and ethyl acetate. For this, 80 g of each methanol extract were dissolved in 200 mL of methanol. To this solution, 200 mL of hexane were added and the mixture gently shaken and the two phases were separated using separating funnel. The upper phase, the hexane fraction, was kept aside while to the lower portion, 200 mL of water and 200 mL of ethyl acetate were added. After shaking, the upper phase constituted the ethyl acetate fraction while the lower phase was the residual fraction. The solvents were evaporated under vacuum at 40°C to give the hexane fraction (Hex), the ethyl acetate fraction (EA) and the residual fraction (Res)[10].

Treatment and seed germination: Stock solutions of methanolic extracts and their fractions were prepared by dissolving separately10 mg of each extract in 800 µL of Tween 80 (surfactant) and the total volume adjusted to 8 mL with sterile distilled water to a final concentration of 1.25 mg mL^{-1}. Test concentrations (0.625, 0.312 and 0.156 mg mL^{-1}) were obtained by serial dilution of the stock solutions. A positive control consisted of a 0.025 µg mL^{-1} naphthalene acetic acid (NAA) from a stock solution of 10 µg mL^{-1} in sterile distilled water, while distilled water was used as negative control.

The tomato seed germination experiment was carried out using 90 mm petri dishes containing two layers of Whatman papers No. 2. About 10 mL of test solutions were used to moisten these papers. Seed germination was monitored by looking for a visible protrusion of the radicle from day 1 of experiment onwards. All sets of treatments and controls were prepared in triplicate of 10 seeds and the experiment was repeated 3 times. The experiment was carried out at 25±2°C and12 h daylight.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) test and RSa_{50} of extracts determination: The free radical scavenging activity of the seedling extracts was determined using DPPH methods described by Blois[11]. About 100 µL of extract or fraction solution (2000 µg mL^{-1}) were introduced into the tubes of the first line. Then 100 µL of methanol were added into all tubes from the second line, followed by successive two fold serial dilutions. Finally, 900 µL of a methanolic solution of DPPH (20 mg L^{-1}) was added in the first 3 series and 900 µL of pure methanol in the last one. After 30 min in dark at room temperature (22±2°C), the absorbance of the contents of each tube were read with a spectrophotometer at 517 nm. This absorbance was converted into scavenging percentages according to the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of DPPH - Absorbance of test solution}}{\text{Absorbance of DPPH}} \times 100$$

The obtained radical scavenging percentages were plotted against the logarithmic values of the concentrations. From this linear regression curve, the radical scavenging activity fifteen (RSa_{50}), corresponding to the amounts of extract/fraction necessary to decrease by 50% the free radical DPPH were determined.

Total phenol determination: The total phenol contents (TPC) of seedling extracts were analyzed using the Folin-Ciocalteu method[12]. The reaction mixture consisted of 0.02 mL of extract (2 mg mL^{-1}), 0.2 mL of a 2N Folin-Ciocalteu reagent and 0.4 mL of a 20% sodium carbonate solution. This mixture was stirred and incubated in a water bath at 40°C for 20 min and then the absorbance was read at 760 nm. A gallic acid solution (0-2 mg mL^{-1}) was used as standard to obtain a calibration curve. The results were expressed in milligrams equivalent of gallic acid per gram (mEq g^{-1}) of extract or fraction.

Total protein content determination: For each sample, 0.5 g of powdered tomato seedlings was digested in a Kjeldahl digestion flask containing 10 mL of concentrated H_2SO_4 and 0.2 g of selenium (catalyst). After 3 h of mineralization, a clear green solution reflecting the conversion of the organic nitrogen to ammonium sulfate was obtained. The flask was then cooled using tap water and its contents transferred to a 100 mL volumetric flask and the volume adjusted using distilled water. Ammonia was steam distilled from the digest into a trapping solution of 20 mL of 40% sodium hydroxide solution. 10 mL of the distillate was collected in a 250 mL conical flask containing 20 mL of 0.1 N boric acid solution and 0.1 N bromocresol green and methyl red solutions as indicators. A volume of 150 mL (Ve) of distillate was recovered and titrated with a 0.01 N HCl solution (V). The nitrogen (N) content was then calculated according to the following formula:

$$\text{N (DM\%)} = \frac{(V - V_0) \times 100 \times 0.14 \times 10^{-3}}{m \times V_e} \times 100$$

V = Volume of HCl used for sample titration
V_0 = Volume of HCl used for white titration
V_e = Volume of the mineralized solution used for the distillation
m = Mineralized sample weight
DM = Dry matter

Qualitative chemical screening of plant extracts

Phenol test: About 100 mg of plant extracts were dissolved in 3 mL of ethanol and 3 drops of the 10% $FeCl_3$ solution added. The appearance of a blue-violet or greenish color indicated the presence of phenols[13].

Alkaloid test: Extract solutions were spotted on TLC plates and eluted with the appropriate solvent system before sprayed with the Dragendorff reagent. The presence of alkaloids was confirmed by the appearance of orange-yellow spots after spraying[13].

Saponin test: About 250 mg of plant extracts were dissolved in a mixture of 5 mL of distilled water and 3 mL of methanol. The mixture was stirred vigorously and the formation of foam of at least 1 cm in height which persisted for 15 min indicated the presence of saponins. For confirmation, the foam formed was mixed with 3 drops of olive oil and then the mixture was stirred. The formation of an emulsion confirmed the presence of saponins[14].

Total flavonoid test: About 250 mg of plant extract were diluted in a mixture of 2.5 mL of distilled water and 1.5 mL of methanol. After homogenization, the mixture was filtered. To 1.5 mL of the filtrate, 0.1 g of magnesium chips and 3 drops of concentrated hydrochloric acid were added. The development of a red orange or pink color indicated the presence of flavonoids[15].

Tannin test: About 100 mg of plant extract were stirred in 2 mL of distilled water. The solution obtained was filtered and then 2 drops of a solution of 3% iron chloride II were added. The appearance of a blue-black or blue-green precipitate indicated the presence of tannins[16].

Anthraquinone test: About 250 mg of plant extract were mixed with 3 mL of benzene, the resulting mixture was stirred and filtered and then 0.5 mL of a 10% ammonia solution was added to the filtrate and the mixture was stirred again. The presence of a pink, red or violet colour in the ammoniacal phase (lower phase) indicated the presence of free hydroxyanthraquinones[16].

Polyphenol test: About 250 mg of plant extract were dissolved in 4 mL of distilled water and then heated in a boiling water bath for 15 min. After cooling, the mixture was filtered. To 1 mL of the filtrate was added 2 drops of a solution of iron cyanide. The occurrence of a blue-green color indicated the presence of polyphenols[15].

Triterpene and sterol test: About 100 mg of plant extract was dissolved in 2 mL of chloroform. Three drops of anhydride acetic acid and 2 drops of concentrated H_2SO_4 were then added. The presence of triterpenoids was characterized by the development of red-brick coloration and that of sterols by the appearance of a blue coloration which then turned to dark green[17].

Anthocyanin test: About 200 mg of plant extract were mixed with 3 mL of a 1% HCl solution. The resulting mixture was boiled. The color change from red-orange to orange-blue indicated the presence of anthocyanins[15].

Statistical analysis: For each extract, at each concentration, the tests were repeated thrice. Data obtained for different parameters (total phenols, total proteins and radical scavenging activities) from the repeated experiments were subjected to multifactorial ANOVA and when this analysis showed significant differences, means were compared in pairs using Duncan's multiple range test at 5% probability level. Pearson correlation was used to evaluate the degree of correlation between total phenol and protein contains for different treatments. The statistical package SPSS 21 (IBM Corporation 1989, 2012, USA) was used for this purpose.

RESULTS

Phytochemical screening of plant extracts: Qualitative screening of phytochemical components revealed the presence of various chemical groups of substances in the tested extracts. These included phenols, alkaloids, total flavonoids, tannins, triterpenes, sterols, anthocyanins and coumarins (Table 1). This chemical composition varied from one plant species to another and for the same plant, from one fraction/extract to another. The water has less extracted the constituents of the plant than the methanol, whatever the plant. This is particularly true for terpenes and sterols and to a lesser extent for saponins, flavonoids and tannins.

Total phenol content of tomato seedling extracts treated with various plants extracts and fractions: The tested extracts and fractions exerted different effects on total phenol contain of tomato seedlings (Fig. 1). This varied with plant

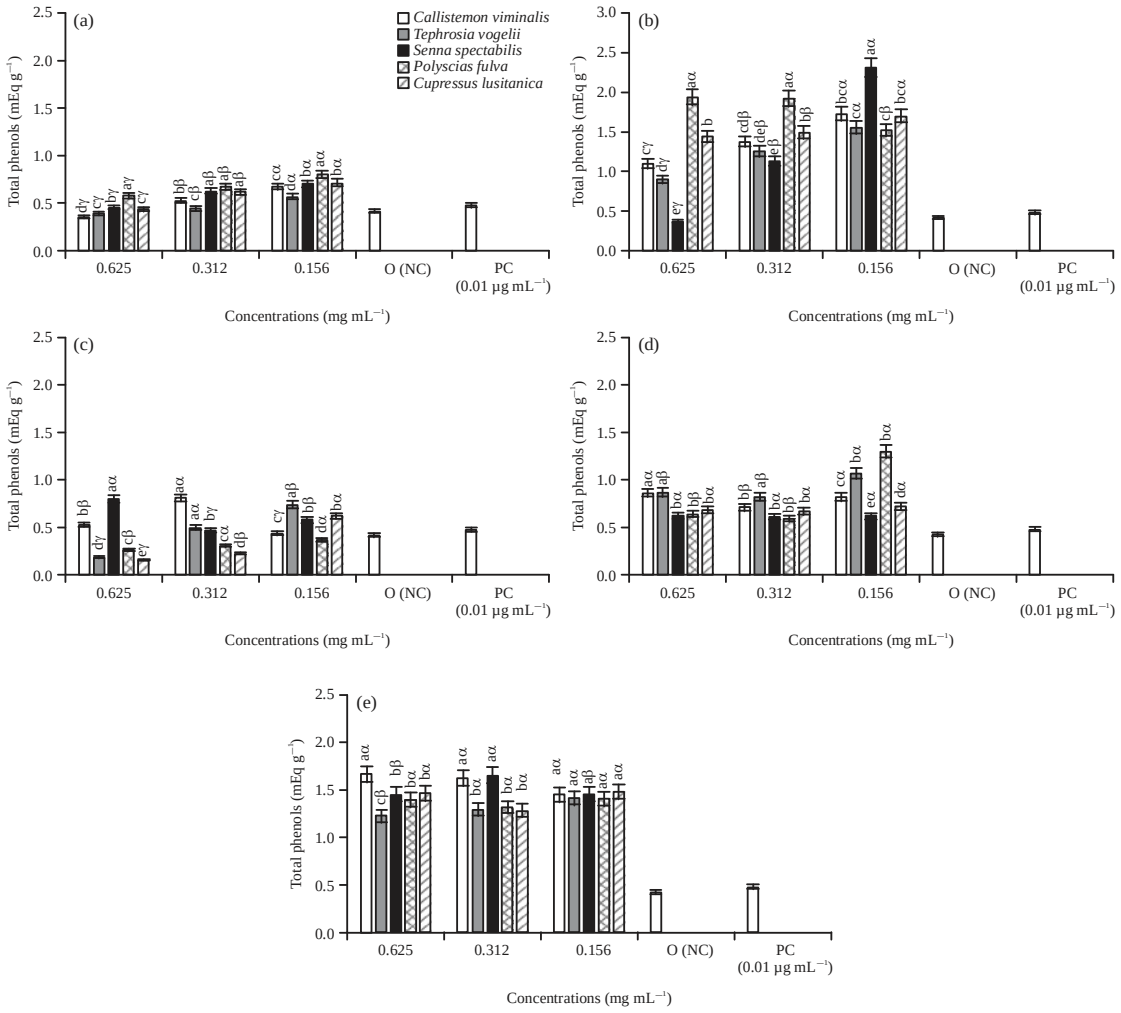

Fig. 1(a-e): Effects of different concentrations of plant extracts and fractions on total phenol contains of tomato seedlings, (a) Aqueous extract, (b) Hexane fraction, (c) Ethyl acetate fraction, (d) Residual fraction and (e) Methanol extract

a,b,c,d,e: Comparison of plant extracts/fractions for the same concentration; total phenol means that are assigned the same letters are not significantly different (Waller-Ducan's test, p>0.05), α,β,γ: Comparison of different concentration for the same plant extract, total phenol means that are assigned the same letters are not significantly different (Waller-Ducan's test, p>0.05), NC: Negative control, PC: Positive control

species and concentrations except for methanol extract. Indeed, methanol extracts, at all concentrations highly stimulated phenol synthesis in tomato seedlings, compared to its fractions and aqueous extracts. No concentration effect was noted for this extract. But it is important to note that the stimulatory effects of hexane fraction of *P. fulva* were significantly higher than that of methanolic extract. The hexane fraction of *S. spectabilis* at 0.625 mg mL^{-1} significantly reduced the total phenol content of seedlings. Aqueous extracts at concentrations 0.312 and 0.156 mg mL^{-1} significantly ($p<0.05$) stimulated the synthesis of phenols as compared to the negative and positive controls, whatever the plant species. The residual fractions also stimulated the synthesis of the phenols than the aqueous extract but less than methanolic extract. For *T. vogelii* and *P. fulva* the stimulatory effects of the residual fraction was greater at 0.156 mg mL^{-1} concentration. Treatment of tomato seedlings with increasing concentrations of ethyl acetate fractions of all the plants generally led to decrease in the total phenol except for *T. vogelii*.

Radical scavenging capacity of tomato seedling extracts treated with various plants extracts and fractions: The methanolic extracts and their residual fractions (except *S. spectabilis*) induced an increase in tomato seedling radical scavenging activity in comparison with fractions and aqueous extracts. Fractionation dispersed this activity in the different fractions. In general, the hexane fractions exhibited the best anti-radical activity (Table 2).

Table 1: Qualitative composition of extracts and fractions from the 5 studied plants

Plants	Phenols	Alkaloids	Saponins	Flavonoids	Tannins	Triterpenes	Sterols	Anthocyanins	Coumarins
C. viminalis									
Aq	+	+	-	+	+	-	-	-	+
MeOH	+	+	+	-	+	-	-	-	+
HexF	+	+	+	-	+	+	-	+	+
EAF	+	+	+	-	-	-	-	-	+
ResF	+	+	+	+	-	+	-	-	+
T. vogelli									
Aq	+	+	-	-	-	-	-	+	+
MeOH	+	+	+	-	-	-	-	-	+
HexF	+	+	+	-	+	-	+	+	-
EAF	+	+	+	+	-	+	-	+	+
ResF	+	-	-	+	-	+	-	-	+
S. spectabilis									
Aq	+	-	-	-	-	+	+	+	-
MeOH	+	+	+	+	-	+	-	+	+
HexF	+	-	+	-	-	-	-	+	-
EAF	+	+	+	-	-	+	+	+	-
ResF	+	+	-	+	-	+	-	-	+
P. fulva									
Aq	+	+	+	-	+	-	-	-	+
MeOH	+	+	+	-	+	-	+	+	+
HexF	+	+	+	-	-	-	-	+	+
EAF	+	+	+	+	-	+	-	+	+
ResF	+	+	+	+	-	+	-	-	+
C. lusitanica									
Aq	+	-	-	-	+	-	-	-	+
MeOH	+	+	+	-	-	+	+	+	
HexF	+	+	+	-	-	+	+	+	
EAF	+	+	+	-	+	+	+	+	
ResF	+	+	-	+	-	+	-	-	+

Aq: Aqueous extract, MeOH: Methanol extract, HexF: Hexane fraction, EAF: Ethyl acetate fraction, ResF: Residual fraction

Table 2: Effects of plant extracts and fractions on tomato seedlings scavenging activity fifty (RSa50 mg mL^{-1})

Plants	*C. viminallis*	*T. vogelii*	*S. spectabilis*	*P. fulva*	*C. lusitanica*	NC-	PC+
Aqueous	$21.6\pm0.9^{c\varepsilon}$	$24.6\pm1.1^{b\gamma}$	$24.3\pm0.8^{b\beta}$	$20.2\pm0.2^{\varepsilon}$	$20.6\pm0.9^{c\varepsilon}$	24.7 ± 1.1^{b}	29.5 ± 1.3^{a}
Aqueous	$21.6\pm0.9^{c\varepsilon}$	$24.6\pm1.1^{b\gamma}$	$24.3\pm0.8^{b\beta}$	$20.2\pm0.2^{\varepsilon}$	$20.6\pm0.9^{c\varepsilon}$	24.7 ± 1.1^{b}	29.5 ± 1.3^{a}
MeOH	$29.2\pm1.0^{b\beta}$	$33.1\pm1.2^{a\alpha}$	$27.2\pm0.8^{c\alpha}$	$32.9\pm0.6^{a\alpha}$	$29.6\pm0.8^{b\alpha}$	24.7 ± 1.1^{d}	29.5 ± 1.3^{b}
Hex	$22.3\pm0.8^{c\gamma}$	$16.1\pm1.0^{f\varepsilon}$	$20.1\pm1.2^{d\gamma}$	$14.3\pm1.2^{e\delta}$	$20.7\pm0.5^{d\varepsilon}$	24.7 ± 1.1^{b}	29.5 ± 1.3^{a}
AE	$28.0\pm1.2^{a\beta}$	$25.2\pm0.9^{c\beta}$	$20.0\pm0.7^{e\gamma}$	$22.1\pm1.2^{d\gamma}$	$26.2\pm1.2^{b\beta}$	24.7 ± 1.1^{c}	29.5 ± 1.3^{a}
Res.	$36.5\pm0.8^{a\alpha}$	$25.1\pm0.6^{c\beta}$	$20.2\pm0.6^{e\gamma}$	$26.2\pm1.5^{c\beta}$	$26.2\pm0.8^{c\beta}$	24.7 ± 1.1^{d}	29.5 ± 1.3^{b}

a,b,c,d,e,f: Comparison of RSa50 of different plant species for the same type of extract/fractions, mean assigned with the same letter are not significantly different ($p<0.05$, Waller-Duncan's test), α,β,γ,ε,δ: Comparison of extracts and fractions of the same plant, mean assigned with the same letter are not significantly different ($p<0.05$, Waller-Duncan's test), NC: Negative control, PC: Positive control

Effects of various plants extracts and fractions on tomato seedling protein content: Aqueous extracts of *C. viminalis*, *S. spectabilis* and *C. lusitanica* at the concentration of 0.625 mg mL^{-1} significantly ($p<0.05$) stimulated the synthesis of proteins in tomato seedlings. This same concentration for *T. vogelii* and *P. fulva* induced a significant reduction in protein accumulation in tomato seedlings. The methanolic extracts of *C. lusitanica* and *P. fulva* less stimulated than the other methanolic extracts and this effect decreased with increasing concentration of the extract. The concentration effect was not observed with *C. viminallis* and *S. spectabilis*. Overall, the best stimulatory effects were observed with the methanolic extracts of *S. spectabilis* followed by *P. fulva*. In general, treatments of tomato seeds with fractions led to a reduction in the level of proteins in resulting seedlings. For all plants, the hexanic fraction had the lowest stimulatory effect at all concentrations. It tended to reduce protein synthesis in tomato seedlings. With the ethyl acetate fraction, the stimulatory effect was observed only with *T. vogelii* and *C. lusitanica* at the concentration of 0.625 mg mL^{-1}. On the other hand, the residual fractions did not stimulate protein synthesis at this concentration, whereas at 0.312 and 0.156 mg mL^{-1} a stimulatory effect was observed with *C. viminallis* and *P. fulva* (Fig. 2).

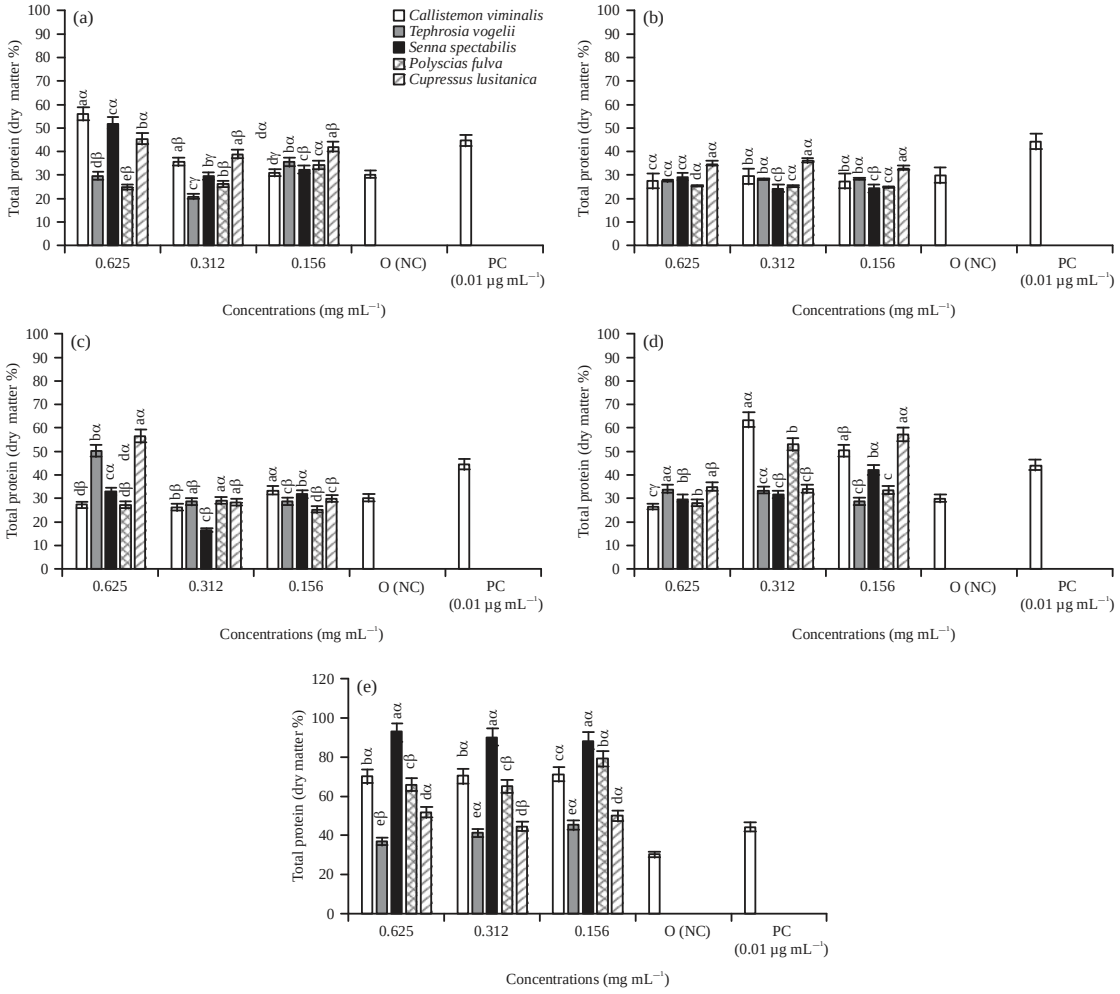

Fig. 2(a-e): Effects of different concentrations of plant extracts and fractions on total protein contents of tomato seedlings, (a) Aqueous extract, (b) Hexane fraction, (c) Ethyl acetate fraction, (d) Residual fraction and (e) Methanol extract

a,b,c,d,e: Comparison of plant extracts/fractions for the same concentration; total phenol means that are assigned the same letters are not significantly different (Waller-Ducan's test, p>0.05), α,β,γ: Comparison of different concentrations for the same plant extract, total phenol means that are assigned the same letters are not significantly different (Waller-Ducan's test, p>0.05), NC: Negative control, PC: Positive control

Table 3: Pearson correlation between protein and total phenol contents of seedlings from seeds treated with various extracts

Plants	Extracts	Pearson's coefficient	Significance
C. vimunalis	aqueous	-0,845**	0,000
	Hex	-0,737**	0,000
	MeOH	,608**	0,007
T. vogelii	Res	-0.773**	0.000
	MeOH	0.581*	0.012
S. spectabilis	Aqueous	0.492*	0.038
P. fulva	Aqueous	0.615**	0.007
C. lusitanica	MeOH	0.509*	0.031

Hex: Hexane fraction, Res.: Residual fraction, MeOH: Methanol extract, **Significant at 0.01, *Significant at 0.05

Correlation between total phenol and proteins contents of seedlings: Protein were negatively correlated to total phenol content in seedlings from tomato seeds treated with aqueous and methanol extracts as well as with hexane fraction of *C. viminalis*. This was also the case with treatment with the residual fraction of *T. vogelii*. In contrast, a positive correlation was noted in seedlings treated with aqueous extracts of *S. spectabilis* and *P. fulva* and seedlings treated with methanol extracts of *T. vogelii* and *C. lusitanica* (Table 3).

DISCUSSION

Qualitative chemical analyses of extracts and their fractions showed that they have different chemical composition. Allelopathy is known to be mainly due to plant secondary metabolites or decomposition products of microbes. These secondary metabolites belong to various

chemical groups such as water-soluble organic acids, long-chain fatty acids, polyacetylenes, benzoquinone, anthraquinone, simple phenols, benzoic acid and its derivatives, cinnamic acid and its derivatives, coumarin, flavonoids, tannins, terpenoids, steroids and alkaloids[9].The differential distribution of these secondary metabolites in extracts and fractions of tested plants may explain difference in their activities, some inhibiting proteins and phenols accumulation while others stimulate their synthesis in tomato seedlings. Indeed, a significant reduction of total phenol content in tomato seedlings was observed after treatment of tomato seeds with ethyl acetate fraction from *P. fulva*, *S. spectabilis* and *C. lusitanica*. This decrease in the total phenol content might have affected chlorophyll contents as it was described with ferulic acid[18]. It might also affect the structure and growth of tomato plant as phenolic compounds are able to influence lignin and pigment synthesis in plants and then influence their development[19]. Phenolic compounds influence growth, photosynthesis, reproduction and many other important processes[20]. For example, it has been reported that ferulic acid, a phenylpropanoid secondary metabolite derived from cinnamic acid, had significant inhibitory effects on chlorophyll, protein and antioxidant enzymes contents of tomato seedlings[21]. It could then be postulate that the difference in total phenols contents of tomato seedlings may be responsible of the differential antiradical scavenging activities in tomato seedlings, these activities being especially known to be linked to phenol content of the plants[22].The reduction of total phenol content in tomato seedlings could also have a negative impact on their resistance to insects, since this type of compound are known to protect plant against insect attacks[23].

Hexane fractions stimulated protein and phenol synthesis in tomato seedlings and this effect was correlated to high antiradical scavenging activities. For the same reason as above, these effects could benefit to tomato seedlings and improve their development. It may be presumed that, subjected to stress of allelochemicals contain in these fractions, the synthesis of specific proteins was stimulated in tomato seedlings to improve their tolerance to stress conditions[24]. This may be the case of antioxidant enzymes that act by reducing the quantity of free radical compounds in the seedlings, materialized by the increase in their antiradical scavenging activities as it is known that synthesis of protein involved in protection against oxidative stress is one of the mechanisms plants use to adapt to stress conditions[25]. Indeed, in tomato as in other plant species, antioxidative enzymes play a key role in protecting the plant against imbalanced production of reactive oxygen species, through their catalytic effects on oxidative phenols and lignin production in response to abiotic stress[26]. In tomato, polyphenol oxidase plays a role in resistance to both cotton bollworm and beet armyworm[27]. It is well known that reactive oxygen species can cause direct damage to membrane lipids, proteins and DNA leading to cell death[19]. This was positively correlated to the phenol content of tomato seedlings in many cases and is in accordance with the findings of Sultana *et al.*[26]. The positive effects on studied biochemical parameters mentioned here is not in accordance with those obtained by Oyeniyi *et al.*[28], who showed that methanolic extract of *Tithonia diversifolia* significantly reduce the protein contents in *Vigna unguiculata* seedlings.

Fractionation of methanolic extract showed positive effect on antiradical scavenging activities with hexane fraction inducing the best antiradical scavenging activities in tomato seedlings suggesting that the active principle in plant extracts are less polar. The total phenol content of seedling extracts obtained from tomato seed treated with ethyl acetate and residual fractions decreased. It may be presumed that having higher extracting power, methanol extracted non-active substances that diluted the active molecules[29] although methanol is widely used as the most effective solvent for extraction of antioxidants and phenolic compounds[30].

Some of the extracts/fractions inhibited proteins and phenols synthesis in tomato seedlings while other stimulated it. Allelopathy is a dual phenomenon, characterized by inhibitory or stimulatory effects of some plant on others in an ecosystem[4]. In this study, higher concentrations sometime inhibited protein and phenol synthesis in tomato seedlings, while the lowest concentration stimulated it. This phenomenon plays a major role in agricultural management. The results suggest that the five plants tested in this study can be useful in the management of tomato crop exploiting their antioxidant and protein synthesis stimulatory effect on tomato[31]. The use of this strategy in management of tomato crop may be achieved through field studies, which should be oriented towards evaluating the insecticidal and herbicidal effects of these extracts on weeds. *T. vogelii* in particular contains rotenoids and essential oils, which are known for their insecticidal properties[32] and field experiment showed that *T. vogelii* mulching increased the biomass of corn by 14.0%[33].Thus, this plant could be used to stimulate the growth of the tomato while fighting against insect attacks.

CONCLUSION

Methanol extracts, hexane fractions and residual fractions of the tested plants generally stimulated the phenol synthesis in tomato seedlings. Moreover, there was an overall

stimulatory effect of methanol extracts on the accumulation of protein in tomato seedlings. These beneficial effects may be useful for improving tomato protection in field, combating oxidative stress, increase plant development and subsequently the productivity of tomato in a controlled system. Further studies, particularly field experiments are needed to define the conditions of use.

SIGNIFICANCE STATEMENT

This study showed that methanol extracts of the 5 plants studied stimulate the synthesis of phenols in tomato seedlings and in some cases the protein level. This was the case in particular for *Tephrona siavogelli*, *Senna spectabilis* and *Polyscia fulva*. The decay of these plants in the soil can release allelochemical substances that may stimulate tomate growth. On the other hand, it would be inadvisable to let *Callistemon viminalis* and *Cupressus lusitanica* grow close to tomato, because of their potentially toxic effects for this plant. The findings of this study will be useful to improve the understanding of the interactions between tomato and the neighboring plants in it environment.

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