Antioxidant and antiproliferative activity of Stachys glutinosa L. ethanol extract

ANTIOXIDANT, ANTIPROLIFERATIVE, AND ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT OF *STACHYS GLUTINOSA* L.

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ABSTRACT

Ethanol extracts of *Stachys glutinosa* L. (Lamiaceae) were investigated for the antioxidative properties, as well antiproliferative action on tumor cells and antimicrobic activities. The antioxidant activities were investigated by 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, 1,1diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, ß-carotene/linoleic acid assay, scavenging of hydrogen peroxide (HRPO test), superoxide anion scavenging (S.A.S. test), and hypochlorous acid scavenging (taurine test). The antioxidant activity was reported as IC_{50} and reveals antioxidant effects. Antiproliferative effects were measured *in vitro* on three cell lines: HepG2 (human hepatocarcinoma), MCF7 (breast adenocarcinoma) and C2C12 (mouse myoblast) cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Extract induces variation of cell viability on all cell lines tested. At 200 µg/ml, the effects on cell viability result -23, -27, -37%, respectively for C2C12, MCF7 and HepG2). Ethanols extract exhibits also antibacterial activity against *E. coli* and *S. pyogenes*.

Introduction

It is well known that oxygen is essential for many living organisms. In contrast metabolites of oxygen (reactive oxygen species ROS), like free radicals are potentially harmful and can give rise to so called oxidative stress [A]. Organisms defends them-self against ROS by different antioxidant systems, mainly identified as enzymes, vitamins, trace elements and natural products that can be introduced as food, by diet, or as

nutraceutics. [B, C] A large literature shows that wild flora still represent a promising source of plant extracts and phytochemicals of antioxidant activity [D, E, F].
After evidence of potential dangerous effects for health from synthetic antioxidant, such as of as tert-butylhydroquinone and tert-butyl-4-hydroxyanisole, nowadays a renewed interest is observed for medicinal plant with antioxidant activity as potential food additives [G]
The antioxidant activity of plant extracts is mainly due to substances known as phenolic compounds. The antioxidant activity is often directly related to the presence of this biomolecules in plants and extracts. [H]

Stachys glutinosa L.(Lamiaceae) is a fruticose dwarf shrub, widespread in Sardinia, Corsica and Capraia Islands [1], very common on different substrata from the sea level to higher mountain. The whole plant is covered by weak thorny stems emanating an unpleasant smell [2]. The plant is used for medicinal purposes (mainly as antispasmodic and antiseptic) in folk medicine [3]. The phytochemical composition of this specie results marginally studied. The compositions of the essential oil of *S. glutinosa* from Corsica [4, 5] and those obtained from wild plants collected in Sardinia were studied [6] The iridoidic composition of *Stachys glutinosa* L. was also examined and the presence of the known harpagide and acetyl-harpagide were showed together with that of a new di-glycosidic iridoid, the 5-allosyloxy-aucubin. The presence of allose in *Stachys* genus seems to be a chemotaxonomical character [7]. The biological activity of this specie result unexplored, except for a antimicrobial screening that reveal activity against *Vibrio cholerae*, *Candida* strains and *Rhodotorula rubra*. In order to evaluate the potential biological activity of *S. Glutinosa*, the ethanolic extract was tested for antioxidant, antiproliferative and antimicrobic activities.

2. Materials and methods

2.1 *Chemicals*

All chemicals were obtained from Sigma-Aldrich Co (St. Louis, MO) and the solvents were from Merck (Darmstad, Germany) unless otherwise indicated. All of the reagents were prepared in distillated water.

2.2 *Plant material*

Stachys glutinosa was collected in the flowering period (April-July 2010), from Santa Maria's Island (Sardinia). A specimen has been deposited in the Herbarium SASSA at the N° 1099 in the Department of Chemistry and Pharmacy, University of Sassari. Aerial part of plant were cut into small pieces and powdered. The plant material was extracted in ethanol by maceration. The extract was filtered, evaporated to dryness in vacuum and stored in the dark at -20°C, until use.

2.3 *Total phenolics*

A derived method of Folin-Ciocalteu, according to Singleton et al. (1965) [8] was used: 7 ml distilled H_2O, 0.5 ml Folin-Ciocalteu reagent and 0.5 ml of extract were mixed. After 3 min, 2 ml of 20% Na_2CO_3 were added and heated at 100°C during 1 min, in a water bath, comparatively to a gallic acid standard. Absorbance was measured at 685 nm after cooling in darkness and the results expressed in mg of gallic acid/100 g sample. All values were uniformly expressed referring to corresponding dry extract weight. All measures were repeated three times and averaged.

2.4 Flavonoids content

The flavonoids content was estimated by the $AlCl_3$ method: 1 ml of ethanol extract solution was added to 1 ml of 2% ethanol $AlCl_3 \cdot 6H_2O$. The absorbance was measured 10 min later at 430 nm comparatively to a rutin standard. The results were expressed in mg rutin/100 g sample. All values were uniformly expressed referring to corresponding plant dry weight. All measures were repeated three times and averaged [9].

2.5 Total flavanols content

The flavanol content was expressed in mg of (-) epicatechin:100 g sample by comparison with standard (-) epicatechin treated in the same conditions. One milliliter of ethanol extract was added to 5 ml of 0.1% (w:v) *p*-dimethylaminocinnamaldehyde in ethanol : HCl (3:1; v:v) reagent. The absorbance was measured 10 min later at 640 nm. All values were uniformly expressed referring to corresponding plant dry weight. All measures were repeated three times and averaged [10].

2.6 Oligomeric proanthocyanidin content

The proanthocyanidin content was expressed in mg of cyanidin chloride/100 g sample after heating the extracts in *n*-butanol : HCl following the method of Porter et al. (1986) [11]: 0.5 ml of ethanol extract, 6 ml of *n*-butanol : HCl (95:5; v:v) and 0.2 ml of 2% (w:v) solution of $NH_4Fe(SO_4)_2$, $12H_2O$ in 2 M HCl were mixed. The tightly capped tubes were heated during 40 min at 92°C in a water bath. After cooling, the colored solutions were measured at 550 nm. All values were uniformly expressed referring to corresponding plant dry weight. All measures were repeated three times and averaged.

2.7 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

The antioxidant capacity assay was carried out using the improved 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid $(ABTS)^{\cdot+}$ radical cation decolorization assay as described by Re et al. (1999) [12]. $ABTS^{\cdot+}$ radical cation was generated by oxidation of ABTS with potassium persulfate. ABTS was dissolved in deionized water to 7 mM concentration, and mixed with 2.45 mM potassium persulfate. The reaction mixture was left to stand at room temperature in the dark for 12–16 h before use. The $ABTS^{\cdot+}$

solution was diluted with ethanol to an absorbance of 0.700±0.020 at 734 nm. Then variable amounts of ethanol extract were added to ABTS solution to a total volume of 1ml and allowed to react for 5 min. Control (without sample) was used as blank. Trolox was used, as reference antioxidant substance (ranging concentration 0-15 µM). Appropriate solvent blanks were run in each assay. The activity was calculated as 50% inhibition concentration (IC_{50}). All determinations were carried out in triplicate and averaged.

2.8 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The antioxidant activity was determined also by the DPPH radical-scavenging method according to Peterson and Hahn (2002) [13]. 100 µl of ethanol extract of sample at different concentration was mixed with 900 µl 100 mM Tris-HCl buffer, pH 7.4, and then added to 1 ml 0.5 mM DPPH in ethanol (250 µM in the reaction mixture). The control sample was prepared using ethanol. Trolox was employed as a standard antioxidant to compare the radical-scavenging activities. Absorbances of the mixtures were measured at 517 nm. The activity was calculated as 50% inhibition concentration (IC_{50}). All determinations were carried out in triplicate and averaged.

2.9 ß-Carotene/linoleic acid assay

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. A stock solution of ß-carotene/linoleic acid mixture was prepared as follows: 0.5 mg ß-carotene was dissolved in 1 ml of chloroform, then 25 µl linoleic acid and 200 mg Tween 40 were added. Then, 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) was added with vigorous shaking; 2.5 ml of this reaction mixture were dispensed into test tubes and 10 µl portions of the ethanol extract at different concentrations were added; the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with the synthetic antioxidant butylated hydroxytoluene (BHT) as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. The activity was calculated as 50% inhibition concentration (IC_{50}). All determinations were carried out in triplicate and averaged [14].

2.10 Scavenging of hydrogen peroxide

The H_2O_2 was measured according to previously published methods [15, 16]. Briefly, 10 µl of ethanol extract of the sample at different concentration were added to 100 µl H_2O_2 in a final volume (1 ml) of PBS, pH 7.4. After 15 min at 37°C, 1 ml phenol red (0.2 mg/ml) containing horseradish peroxidase (17 U/ml) was added; 15 min later, 50 µl 1N NaOH was added and the amount of H_2O_2 was determined by measuring the absorbance at 610 nm. Trolox was used as the positive control. The activity was

calculated as 50% inhibition concentration (IC_{50}). All determinations were carried out in triplicate and averaged.

2.11 Superoxide anion scavenging ($^{\bullet}O_2^-$)

The scavenging of ($^{\bullet}O_2^-$) were assayed by measuring the inhibition ability of the nitroblue tetrazolium (NBT) reduction caused by ($^{\bullet}O_2^-$) generated by the xanthine oxidase/hypoxanthine system. Reduction of NBT to formazan was measured as the change in absorbency at 560 nm versus time (3 min) either in the absence of ethanol extract of sample or with increments of sample concentrations. Briefly the reaction mixture (1 ml) contains the following reagents: phosphate buffered saline (PBS, 10 mM phosphate, NaCl 150 mM, pH 7.4); hypoxanthine (2mM); xanthine oxidase (3 units); and NBT (1.2 mM). Trolox was used as the positive control. The activity was calculated as 50% inhibition concentration (IC_{50}). All determinations were carried out in triplicate and averaged [17, 18].

2.12 Hypochlorous acid scavenging (HOCl)

The amount of HOCl was measured by the chlorination of taurine. Briefly, 100 µl sodium hypochlorite (600 mM) were added to 100 µl taurine (150 mM) and 10 µl ethanol extract of sample at different concentration in a final volume of 1 ml PBS, pH 7.4. Absorbance was measured at 350 nm after the addition of 10 µl 2 M potassium iodide. Trolox was used as the positive control. The activity was calculated as 50% inhibition concentration (IC_{50}). All determinations were carried out in triplicate and averaged [19-21].

2.13 Cytotoxic activity

Antiproliferative effects were measured *in vitro* on three cell lines: HepG2 (human hepatocarcinoma), MCF7 (breast adenocarcinoma) and C2C12 mouse myoblast cell line by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, a limited number of human cancer cells (2500/well) were seeded onto a 96-well microplate and incubated overnight. After 24h, the original medium was removed and 200 µL of new medium containing the sample (100 and 200 µg/ml) was added. After incubation for 24h and 48h the amount of living cells was assayed by the addition of 20 µl of 5 mg/ml MTT in medium. After 3h MTT was converted, in intact mitochondria, by reductase and precipitated as blue crystals. The medium was then removed, and the precipitated crystals were dissolved in 200 µl of dimethyl sulfoxide (DMSO), and after 30 min the amount of reduced MTT was determined at 540 nm (Varian50 MPR, Microplate reader) as already described [**L**]. Results are expressed as mean variation percentage of cell viability compared to control untreated groups.

2.14 Antimicrobial activity

Extract of *S. glutinosa* were tested against different microorganism strains from American-Type Cell Culture (ATCC) reference bacteria as well as clinical isolates: 2 Gram negative bacteria (*Pseudomonas aeruginosa* (DSM 1117), *Escherichia* coli (ATCC 13706) and 4 Gram positive bacteria (*Streptococcus agalactiae* (ISS1), *Streptococcus pneumoniae* (ISS3), *Streptococcus pyogenes* (ISS2), *Staphylococcus aureus* (ATCC 25923). Antimicrobial activity was evaluated using the agar disk diffusion method. [22] [23] Bacterial strains were cultivated on Müeller Hinton broth (Oxoid, Unipath Limited, Basingstoke, UK). Cell cultures were incubated at 37°C for 24 hours before test. The cell suspension were adjusted with sterile saline solution to obtain a turbidity comparable to that of McFarland n. 0.5 standard (1.5 x 10^8 cells/ml). Müeller-Hinton agar plates were spread with 1 ml each microbial suspension, then the excess suspension was aspirated. Petri dishes of Sabouraud dextrose agar were used for yeasts. Sterile paper disks (5.0 mm in diameter) were impregnated with 10 µl test solution at concentrations 1-0.5 mg/ml and placed on the inoculated plates. Chloramphenicol were used as positive controls (30 µg). A negative control, inoculating same volume of DMSO without extract, was also included. All the experiments were conducted in triplicate and results were expressed as mean values.

2.15 Statistical analysis

Analysis of variance and significances were performed by One-way ANOVA with Newman-Keuls Multiple Comparison post test, using GraphPad Prism version 5.00 (GraphPad Software, San Diego, California USA).

3. Results and Discussion

3.1 Chemical components

S. glutinosa dried extract contains total phenols (93.56±3.62 mg/g as gallic acid equivalents), proanthocyanidins (21.02±1.78 mg/g calculated as cyanidine chloridrate), total flavonoids (15.21±2.05 mg/g, as rutin) and total flavonols (6.7±1.20 mg/g, as (-) epicatechin), as reported in Table 1. Results were expressed as mean value of three replicates and were referred to extract dry weight. Quantitative data confirm that this specie can be an interesting source of equilibrated mix of different polyphenols, also if the total amount is not one of the highest, almost referred to the genus *Stachys*. To the best of our knowledge, no references are available for the title plant on phenolic composition. In Kukić (2006) [24] are reported the total phenolic data of four species, *S. anisochila*, *S. beckeana*, *S. plumosa* and *S. alpina* ssp. *Dinarica*, that result in a ranging concentrations 65-192 mg/g of dry extract. Total phenol content in different species (*S. setifera* C. A. Mey. *S. inflata*, *S. persica*, *S. byzantina*, *S. laxa*, *S. turcomanica*, *S. subaphylla* and *S. trinervis*) result in ranging concentration from 4.3 (in *S. trinervia*) to 44.5 (in *S. fruticulosa*) mg/g dried plant. [25].

3.2 Antioxidant activity

Ethanol extract of *S. glutinosa* were screened for antioxidant activity by six test systems: ABTS assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, ß-carotene/linoleic acid assay, scavenging of hydrogen peroxide, superoxide anion scavenging ($^{\bullet}O_2^-$) assay, and hypochlorous acid scavenging (HOCl) assay. Table 2 reported the antioxidant activity of ethanol extract of *S. glutinosa.* The chemical complexity of ethanolic extracts, often a mixture of dozens of compounds with differences in functional groups and chemical behaviour, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among different ways in use to report the antioxidant activity, the amount of sample necessary to reduce the oxidation by 50 % (IC_{50}) was used. The in vitro antioxidant activities of the extract tested were compared to those of Trolox or BHT, widely used as reference drugs in literature.

ABTS and DPPH radical scavenging methods are common spectrophotometric procedures for determining antioxidant capacities of components [26]. DPPH radical involves a hydrogen atom transfer process, and ABTS radical involves an electron transfer process [27]. The antioxidant activity on DPPH and ABTS radicals of *S. glutinous* may be attributed to a direct role in trapping free radicals by donating hydrogen atom or electron.

Dry extract was diluted in ethanol at 1 mg/ml, and the solution directly tested. As reference antioxidant a ranging concentration of trolox solutions were used, in order to determine a range for linear response. The IC_{50} activity of extract at 1mg/ml results 2.4±4 (ABTS assay) and 2.0±3 (DPPH assay) expressed as mg of Trolox equivalents. Compared with the reference, *S. glutinosa* is about twice time less active. Lipid peroxidation may be described as the oxidative deterioration of unsaturated fatty acids caused by free radicals and the changes resulting from this process. ßcarotene/linoleic acid assay were chosen as the test for the determination of ethanolic extract of *S. glutinosa* antioxidant activity on polyunsaturated fatty acids. The autooxidation of linoleic acid in the negative control group increased rapidly on day 1, and reached maximum level on day 4. In our study, *S. glutinosa* significantly inhibited the degree of lipid peroxidation. The activity may be due to termination of the radical chain reaction after its propagation by scavenging highly toxic lipid peroxy radical as the curve conc/lipid peroxide inhibition suggests. The IC_{50} activity of extract results 12±3 expressed as mg of BHT equivalents. Compared with the reference, *S. glutinosa* is less active, but owing the high specificity of BHT there was an high lipid peroxidation. The activity in lipid peroxidation test results higher than Trolox (0.1 ± 0.02 mg_s/mg_r). We also investigated the ability of ethanol extract to scavenge in vitro oxygen-derived species, such as hydrogen superoxide anion ($^{\bullet}O_2^-$), peroxide (H_2O_2), and hypochlorous acid (HOCl) (which are known to be released, among other mediators, by activated phagocytic cells during inflammatory disorders). Both ($^{\bullet}O_2^-$) and H_2O_2, like HOCl and OCl^-, are powerful oxidants capable to react with amines, amino acids, sulphydryls, disulfides, thioethers, aromatics and a variety of potentially important biological substrates. For this reason, the discovery of some compounds with excellent hydroxyl radical scavenging ability would be significant for some ailments induced by oxidative

stress. Superoxide anion is an oxidant that can generate more dangerous species, including singlet oxygen and hydroxyl radicals, also hydrogen peroxide may induce hydroxyl radicals, which would result in great damage to cells. Hydroxyl radicals are highly strong reactive oxygen species, and there is no specific enzyme to defense against them in human body [28-30]. Horseradish peroxidase (HRPO), S.A.S., and taurine assays were chosen for the determination of antioxidant activity on hydrogen peroxide (H2O2), ($^{\bullet}O_2^-$) and hypochlorous acid (HOCl). The ethanolic extract revealed an antioxidative effects, showing IC_{50} values in the range 0.17-0.22 mg: 0.22$\pm$ 0.05 (HRPO assay), 0.24+0.06 (S.A.S. assay), and 0.17+0.04 (taurine assay) (Table 2). The extent of the effects can be compared to that of trolox, 1 mg trolox being equivalent to 1.1-3.2-2.5 respectively. In the test, *S. glutinosa* showed relevant scavenging activity on the three reactive oxygen species that can be, at least in part, justified by polyphenols presence in the extract.

3.3 Cytotoxic activities

In figures 1-3 were reported data of percentage inhibition of cells treated with different doses of extract. The extract from *S. glutinosa* was evaluated for *in vitro* cytotoxic activities on tumor (HepG2, human hepatocarcinoma and MCF7, breast adenocarcinoma) and non tumor (C2C12, mouse myoblast) cell lines, by MTT test. Cells were stimulated at two different doses of 100 and 200 µg/ml and cell viability quantified after 24 and 48h. In all experiments extract reduce cell viability in a dose dependent response. The most sensible cell lines results the HepG2, after 24h. The stimulation time influence weakly the cells viability, that result more reduced after 48h. On HepG2 the effect of longer time exposure results as an evident reduction of toxicity. In HepG2 and C2C12 the reduction in viability seems related to the dose of extract, while on MCF7, the higher dose of extract have only weak effect on cell proliferation. The extract showed stronger in vitro cytotoxicity on tumor cell lines, with higher proliferative inhibition at -37% and -27% respectively for HepG2 (24h) and MCF7 (48h) and result less effective against muscle cells C2C12 (cell viability -23%, after 48h). In experiments was detected an inhibition of cell proliferation always below 50%, so the resulting IC_{50} is clearly higher than 200 mg/ml. Comparing to drugs used as reference (IC_{50} of 5-fluorouracil: 15.9 µmol/l and 73-81 µM, respectively for HepG2 [31] and MCF7 [32]), the extract can be considered as a mild antiproliferative agent. More interesting for future perspective results the selective activity that seems reduce the viability only on tumor cell lines. No bibliographic data are available on antiproliferative activity of *S. glutinosa*. Only one report can be related to our experimental conditions. In Háznagy-Radnai, et al. (2008),[33] is reported that extracts from other species of *Stachys* (*S. recta*, *S. palustris*, *S. germanica*, *S. byzantina*), at 10 µg/ml on MCF7, afford a reduction of proliferation in 28-55% range. Compared to data of present work, the antiproliferative activity is similar, but the extract was tested concentration from 10 to 20 times lower. Experimental and bibliographic data confirm *S. glutinosa* as a weak inhibitor of tumor cell proliferation. Further studies will be dedicated to understand the effect of extract on cell metabolism, particularly in

antiradical and antioxidant mechanisms, that are clearly involved in pathogenesys of large number of degenerative diseases.

3.4 Antimicrobial activity

Extract obtained from flowers of *S. glutinosa* were assayed for their antimicrobial activity, by measuring the inhibition zone, in presence of different extract concentrations. Results given in Table 4, show that the ethanol extract exhibites a antibacterial activity against *E. coli, S. agaltiae* and *S. pyogenes*; Less sensible resulted *S. aureus* and *S. pneumoniae*, while no significant activity was detected aginst *P. aeruginosa*.
Further investigations are required to detect the relation between chemical composition and antimicrobial activity of the extract.

4. Conclusions

This research reports on the qualitative profile of S. glutinosa ethanolic extract, as well as previously reported in the literature (Serrilli et al. 2006; Karioti et al. 2010) The main phenylethanol glycosides present were betonyoside, forsythoside and melittoside; caffeoylquinic acid derivatives and chlorogenic acid were identified as the predominant phenolic acids. The majority of the constituents were represented by isoscutellarein derivatives. This extract revealed antioxidative effects, showing IC50 values in the range 0.17– 0.22 mg: 0.22 ^ 0.05 (HRPO assay), 0.24 ^ 0.06 (SAS assay) and 0.17 þ 0.04 (taurine assay). The biological assay of *S. glutinosa* extract showed significant scavenging activity on the three ROS that can be, at least in part, justified by the presence of polyphenols in the extract. The extract from *S. glutinosa* was also evaluated for in vitro cytotoxic activities on tumour (HepG2 and MCF7) and non-tumour (C2C12) cell lines, by MTT test. The extract showed stronger in vitro cytotoxicity on tumour cell lines, with higher proliferative inhibition at 237% and 227%, respectively, for HepG2 (24 h) and MCF7 (48 h). Experimental and bibliographic data confirm S. glutinosa as a weak inhibitor of tumour cell proliferation.

Table 1.

Chemical class components of *S. glutinosa*

Total phenols	Flavonoids	flavonols	Oligomeric proanthocyanidins
mg/g d.e. as gallic acid	mg/g d.e. as rutin	mg/g d.e. as (-)-epicatechin	mg/g d.e. as cyanidin chloridrate
93.56±3.62	15.21±2.05	6.7±1.20	21.02±1.78

Table 2. Antioxidant activity of *S. glutinosa* ethanolic extract

	IC_{50} mg	±S.D.	mg_s/mg_r (mean value)
ABTS assay	0.32	0.06	2.4

DPPH assay	0.28	0.05	2.0
Linoleic assay	0.12	0.03	12
HRPO assay	0.22	0.05	1.1
S.A.S. assay	0.24	0.06	3.2
Taurine assay	0.17	0.04	2.5

A lower IC_{50} value indicates greater antioxidant activity.
mg_s = milligram of ethanolic extract with IC_{50} activity ; mg_r = milligram of Trolox or BHT with IC_{50} activity

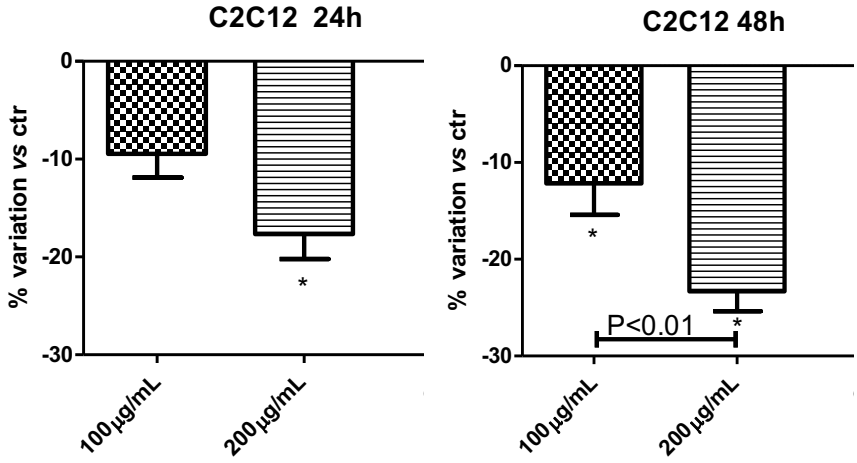
Table 3. Antimicrobial activity of ethanolic extract of *S. glutinosa*

	inhibition zone (in mm)[a]		
Strains	Extract 1mg/ml	0.5 mg/ml	Chloramphenicol [b]
E. coli (ATCC 13706)	12±3	7±2	31±5
P. aeruginosa (DSM 1117)	3±1	2±1	25±4
S. agalactiae (ISS1)	10±4	8±3	30±5
S. aureus (ATCC 25923)	9±3	6±2	35±6
S. pneumoniae (ISS3)	8±2	5±2	25±5
S. pyogenes (ISS2)	13±4	8±3	28±5

[a] Mean of three determinations ± SD.

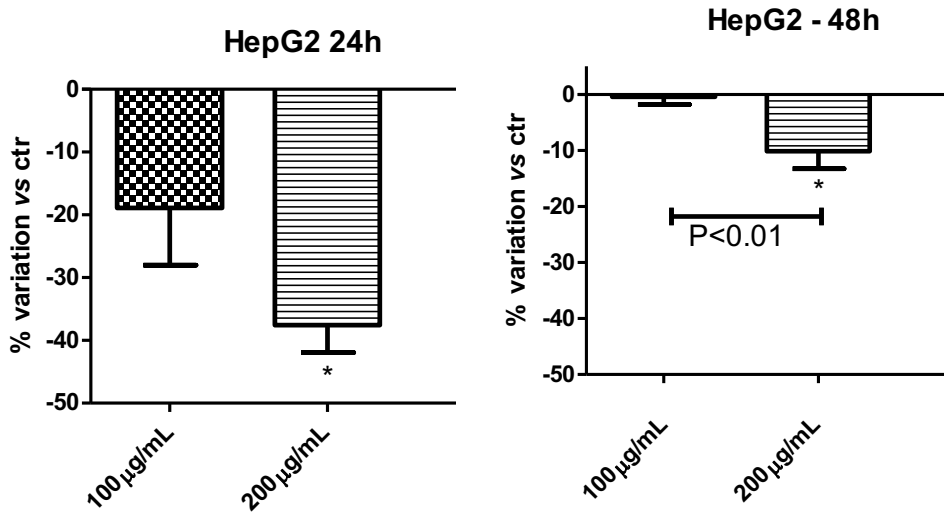
[b] Chloramphenicol (30 μg) was used as positive control for Gram–positive and Gram–negative bacteria; solvent control (DMSO) was negative for all tested strains.

Fig. 1: C2C12 cells viability 24 (a) and 48h (b) after stimulation with *S. glutinosa* extract.



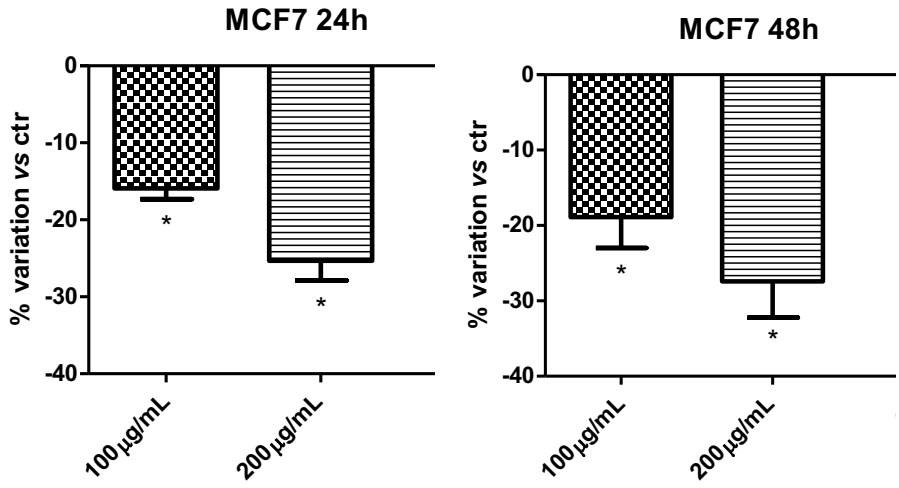
Data are expressed as mean % variation of cell viability *vs* control untreated group. ANOVA P<0.01, *sample *vs* control P<0.01

Fig. 2: HepG2 cells viability 24 (a) and 48h (b) after stimulation with *S. glutinosa* extract.



Data are expressed as mean % variation of cell viability *vs* control untreated group. ANOVA P<0.05, *sample *vs* control P<0.05

Fig.3: MCF7 cells viability 24 (a) and 48h (b) after stimulation with *S. glutinosa* extract.



Data are expressed as mean % variation of cell viability *vs* control untreated group. ANOVA P<0.01, *sample *vs* control P<0.01

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