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Phytochemical and Biological Studies of *Schinus polygamus* Growing in Egypt

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Abstract

The leaf ethanol extract of *Schinus polygamus* (Cav.) Cabrera has evidenced medicinal value as hepatoprotective. It demonstrated inhibitory effects on standard microbes (approximated to) 50% potency of ofloxacin. The same extract evidenced *in vitro* cytotoxicity on human cell lines *viz*, liver carcinoma HEPG2, larynx carcinoma HEP2, and colon carcinoma HCT116 when compared to doxorubicin. The leaf ethanol extract of *S. polygamus* showed variable anti inflammatory, analgesic, and antipyretic activities. Analysis of the hydrolyzed methanol extract of *S. polygamus* by HPLC allowed the identification of four phenolic acids and five flavonoid aglycones. Gallic acid and chlorogenic acid were identified as major phenolic acids. Major identified flavonoid aglycones were luteolin, kaempferol, quercetin, naringenin, and apigenin. The anti-inflammatory, analgesic, and antimicrobial activities support scientifically the use of *S. polygamus* in folk medicine for the fore mentioned variable uses. The content of plant constituents as polyphenols of the leaves of *S. polygamus* justify to some extent the hepatoprotective, anti-inflammatory and analgesic activity with pain tolerance. DNA fingerprinting of the leaves of *S. polygamus* was carried out as a mean of identification of the genetic profile of the Egyptian plant.

Keywords: *Schinus polygamus* Cabrera; polyphenolics; hepatoprotective activity; antimicrobial activity; cytotoxic effect.

Introduction

Plants constitute a rich source of bioactive constituents such as phenolics, terpenoids, coumarins and alkaloids (Harborne, 1993; Stevanovic etal., 2009). With great interest and enthusiasm, many scientific research centers around the world are exploring the medicinal value of plants due to the global belief of their efficacy in treatment. Members of the family Anacardiaceae (Bailey,1953) have long reputation in folk medicine for nutritional value of their edible fruits and seeds, and for variable ailments such as treatment of bowel complain, chronic wounds, pimples, boils, jaundice, hepatitis and relieving inflammatory conditions (Abbasia et al.,2010). *Schinus polygamus* Cabrera, family Anacardiaceae is a tree of about 1.2–2m in height and cultivated for ornamental purpose. Infusion of the leaves of this plant has been used for cleansing of wounds, while the bark decoction that produces a balsamic essence, is prescribed to treat arthritic and feet pains. The latex that emanates from the bark is used as a plaster for pain of muscles. The resin is reported to treat chronic bronchitis (Munoz et al., 1981). Also the aerial part was used as antifertility agent (Montes and Wilkomirsky, 1987). Research on evaluation of plant extracts and their activities necessitates focusing on their crucial phytoconstituents, as well as, their toxicity if any. The presence of well-known flavonoids in *S. polygamus*, namely: kaempferol, quercetin and quercetin-3-*O*-galactoside were reported (Mandich et al., 1984). The hexane

and methanol extract of *S.polygamus* were reported to contain β-sitosterol, quercetin which where associated to analgesic, anti-inflammatory and antipyretic activities (Erazo et al., 2006; Gonzalez et al., 2004). The composition and antimicrobial activity of the essential oil obtained from *S. polygamus* leaves had been reported (Gonzalez et al., 2004). There is an extensive body of literature addressing the escalated distribution of hepatic diseases among the people in Egypt (Lehman, 2008; Strickland et al., 2002)**.** Since the liver is responsible for the breakdown and elimination of most toxic substances, the need for protecting the liver against poisons is a global health problem. Hepatoprotective herbal drugs can offer help by blocking absorption of toxins into liver cells and the formation of inflammatory substances that contribute to liver degeneration. Few reports were traced concerning the analysis of the polyphenolics and flavonoids of the Argentine and the Chilean species and its biological potentiality (Erazo et al., 2006; Gonzalez et al., 2004). However, nothing could be traced on Egyptian *S. polygamus*. The aim of the study is to establish the chemical composition of the polyphenolics and flavonoids of the leaves and to throw light on certain biological activities of Egyptian *S.polygamus*. Furthermore, identification of the plant was achieved by a DNA profiling.

Material and Methods

Plant material

Samples of the leaves of *S. polygamus* Cabrera were obtained from trees growing in El Orman botanical garden, Giza (harvested in May and June 2007) and identified by the Botany Department, Faculty of Science, Cairo University, Giza, Egypt. Sample of the plant is deposited at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Samples of leaves, were stored at -70^{o}C, freeze-dried and ground to a fine powder using a coffee grinder prior to DNA isolation.

Extraction

The air-dried powdered leaves of *S. polygamus* (1 kg) were extracted at room temperature by percolation with 95% ethanol (2 x 1 L). The ethanol extract was evaporated under reduced pressure to give about 260 g greenish brown semi-solid residue (Mabry et al., 1970; Claus and Tylor, 1967; Egyptian pharmacopeia, 2005). The dried residue was suspended in distilled water and successively partitioned between hexane, chloroform, ethyl acetate and 1-butanol saturated with water. The solvent in each case was completely evaporated under reduced pressure to yield 31.1g, 7.5g, 122.2g and 24.5g of dry extract, respectively.

Material and apparatus for phytochemical study

High performance liquid chromatography (HPLC) was performed for qualitative and quantitative analyses of the phenolic constituents on a Hypersil ODS column (particle size 5 µm, 250 mm×4.6mm). Using acetonitrile/15% aqueous acetic acid (40:60v/v) as mobile phase at a flow rate of 1ml/min. Eluted peaks were detected at λ=280,330 nm for phenolics and flavonoids respectively. HPLC apparatus, Agilent Series 1100 apparatus (Agilent, USA) equipped with Quaternary pump, series 1100; deggaser, series 1100; column heater series 1100 and UV detector, series 1100 was used for analysis of poly phenols (Food Technology Research Institute, Giza). Standards of flavonoid aglycones: quercetin, kaempferol, luteolin, naringenin, apigenin, and phenol acids: gallic, chlorogenic, caffeic and ferulic acids were obtained from Fluka, Sigma, Germany. Quantitation of the major flavonoid and phenolic acid constituents in the leaves of *S. polygamus* was determined by HPLC adopting the procedure of (Mattila et al., 2000).

Sample preparation

One gram of the air-dried leaves of *S. polygamus* was weighed into a 100 ml conical flask then dispersed in 40 ml of aqueous methanol (62.5%). The mixture was ultra sonicated for 5 min. To this extract 10 ml of 6 M HCl were added. The flask containing the mixture was placed in a shaking water bath at 90 °C for 2 hours. After hydrolysis, the sample was allowed to cool, filtered, made up to 100 ml with methanol, and ultra sonicated again for 5 min. The sample was filtered through a 0.4 µm membrane filter into the sampler vial for injection.

Standard preparation

The major components of the samples were identified by comparing their retention times to those obtained for the standards (prepared as 10 mg/50 ml solutions in methanol and they were diluted to make concentrations (20-40µg/ml). Quantification was based on measuring the peak areas of both standards and samples by adopting the external standard method (expressed as mg/100g dry weight).Results were the average of

triplicate experiments and are recorded (Table 1).

Biological study

Preparation of the extracts

Ten g of the dried ethanol extract previously prepared was dissolved in distilled water containing few drops of Tween 80 to yield a concentration of 5 % w/v. The leaf ethanol extract of *S.polygamus* was tested for its hepatoprotective, anti inflammatory, analgesic, and antipyretic activities.

Chemicals and kits

Carrageenan: Sigma Co.(0.1 ml of 1% solution, to induce inflammation), indomethacin: Epico, A.R.E.(20 mg/kg body weight [b. wt.], standard anti inflammatory), Brewers dry yeast: Rehab food company (1 ml/100g b. wt. of 40% suspension by intramuscular injection to induce hyperthermia), paracetamol (Paramol): Misr, Mataria, Cairo (20 mg/kg b. wt., standard antipyretic), dipyron-metamizol (Novalgin): Hoechst Orient, Cairo (50 mg/kg b. wt., standard analgesic), carbon tetrachloride (Analar, El-Gomhoreya Co., Cairo, Egypt, for induction of liver damage (5 ml / kg of 25% CCl_4 in liquid paraffin, IP), Silymarin (Sedico Pharmaceutical Co., 6 October City, Egypt, standard hepatoprotective drug (25 mg / kg b. wt.), biodiagnostic kits for assessment of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase enzymes (ALP). Doxorubicin (10mg Adriamycin hydrochloride, in 5ml IV injection, Pharmacia, Italy).

Experimental animals

Adult male albino rats (130-150 g) were obtained from the animal-breeding unit of National Research Center, El-Dokki, Giza, Egypt. All animals were fed on a standard laboratory diet under hygienic conditions and water supplied ad lb.

For testing the effect on the liver, 50 adult male albino rats (130-150 g) were used and were divided into five groups (each of 10). The ethanol extract of the leaves of *S. polygamus* was tested for its hepatoprotective activity using silymarin as a reference drug. The tested extract was administered at a daily dose of 100 mg/kg b.wt.for one month before induction of liver damage (Klassan and Plaa, 1969). Administration of the tested solution was continued after liver damage for another one month. Doses of the drugs were calculated and administered orally by gastric tube (Paget and Barnes, 1964).

Measurement of aspartate aminotransferase ,alanine aminotransferase and alkaline phosphatase enzymes serum levels.

Serum levels of AST, ALT and ALP enzymes were measured in each group at zero time, after one month of receiving the tested drug, 72 hours after induction of liver damage and after one month of treatment with the tested samples(Thewfeld,1974;Kind and King, 1954).

For testing anti-inflammatory, analgesic and antipyretic activities, animals, in each case, were divided into 3 groups, each of ten. The first group was considered as a control, the second was given the appropriate standard drug, and the third was administered 100 mg/kg b. wt of the tested extracts orally.

Determination of median lethal dose (LD_{50})

LD_{50} of the ethanol extract for 24 hours was determined according to the reported procedures (Karber, 1931).

Acute anti inflammatory activity

Acute anti inflammatory effect was determined according to the published procedures (Winter et al., 1962). The percentage of oedema inhibition (% of change) was calculated.

Analgesic effect

The analgesic effect was evaluated according to standard methods (Charlier,1961) by using electric current as a noxious stimulus where electrical stimulation was applied to the rat tail by means of 515 Master shocker (Lafayette Inst. Co.) using alternative current of 50 cycles/sec for 0.2 second. The minimum voltage required for the animal to emit a cry was recorded after one and two hours of oral administration of the tested dose. The percentage of change was calculated.

Antipyretic effect

The induced rise of temperature of rats was recorded at zero time and in the treated groups after one and two hours (Bush and Alexander, 1960). The percentage of change was calculated and the results are recorded.

Statistical analysis

All data were expressed as mean ± SE and the statistical significance was evaluated by the student's "t" test (Snedecor and Cochran, 1967).

In vitro screening for cytotoxic activity

Human tumor cell lines: liver carcinoma (HEPG2), colon carcinoma (HCT116) and larynx carcinoma (HEP2) cell lines, maintained in the laboratory of Cancer Biology Department of National Cancer Institute, Cairo, Egypt were used. The ethanol extract at different concentrations (0-10 µg/ml) in DMSO were tested for cytotoxicity against the fore mentioned human tumor cell lines adopting sulforhodamine B stain (SRB) assay (Skehan et al., 1990). The relation between survivals and the extract concentration was plotted to get the survival curve of each tumor cell line after the application specific concentration. The results were compared to those of the standard cytotoxic drug, Doxorubicin (10 mg Adriamycin hydrochloride, in 5 ml IV injection, Pharmacia, Italy) at the same concentrations were used as standard anti-tumors. The dose of the test solutions which reduces survivals to 50% (IC_{50}) was calculated.

Testing of the antimicrobial activity

The antimicrobial activity was performed against selected bacterial and fungal strains of standard properties. These were maintained in the Micro Analytical Center, Faculty of Science, Cairo University. The tested Gram positive bacteria were [*Bacillus subtilis* ATCC 6051, *Streptococcus faecalis* ATCC 19433 and *Staphylococcus aureus* ATCC 12600]. The Gram negative bacteria included [*Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 10145 and *Neisseria gonorrhea* ATCC 19424], and fungi [*Candida albicans* ATCC 26555 and *Aspergillus flavus*]. Bacteria were grown on nutrient agar (Oxoid, England) and fungi on Sabouraud's glucose agar (Oxoid, England). The ethanol extract was tested against the selected strains at concentration of 20 mg/ml adopting the disc agar diffusion method (Lorian, 1991). Discs impregnated with Ofloxacin and Fluconazole were used as antibacterial and antifungal standards, respectively. Test solution was prepared by dissolving in DMSO at a concentration of 20 mg/ml; aliquots, 10µl each were aseptically transferred into sterile discs of Whatmann filter paper 8 mm diameter.

Material for DNA mapping

- **Buffers***:* Extraction buffer: 0.7 M NaCl, M Tris (pH 7.5), 0.01 M EDTA, 1% (w/v) N-cetyl-N, N, N-trimethylammonium bromide (CTAB), 1% (v/v) β-mercaptoethanol (added immediately before use), washing buffer 1:76% ethanol, 0.2 M Na-acetate, washing buffer 2:76% ethanol, 10 m M NH_4 O-acetate, TE-buffer 10 m M tris (pH 8.0), 1m M EDTA,10x reaction-buffer 100 m M Tris (pH 8.3), 500 m M KCl, 0.01% (w/v) gelatin, Chloroform/ isoamyl alcohol 24:1 (v/v), isopropanol, d NTP (Pharmacia, Sweden), Taq DNA polymerase (Perkin-Elmer / Cetus, USA, Advanced Biotechnologies, UK).
- **Primers**: Five primers were used for randomly amplified polymorphic DNA (RAPD) analysis obtained from Operon Technologies Inc., Almeda, California, USA with the following sequence: A-13;{CAGCACCCAC}, A-14;{TCTGTGCTGG}, A-15;{TTCCGAACCC}, B-01;{GTTTCGCTCC},C-20.;{ACTTCGCCAC}.
- **Molecular weight marker:** 100 bp ladder, Promega Corporation, Madisson, USA.
- **Apparatus:** DNA thermocycler (Hybaid PCR Express) used for amplification of DNA, agarose gel electrophoresis tool (Biorad Wide Mini Sub Cell) used for separation of RAPD fragments according to size and UV Polaroid camera used for visualization of RAPD fragments.

DNA extraction and quantification

DNA was extracted using CTAB method (Doyle and Doyle, 1987). Fifty mg of frozen leaf were powdered in liquid nitrogen, extracted with 0.8 ml CTAB, precipitated with isopropanol. The precipitate was washed in 70% ethanol and dissolved in deionized water.

Amplification of RAPD markers

The polymerase chain reactions were carried out with 100 ηg of genomic DNA template following a thermal cyclic program. Amplified products were analyzed by electrophoresis on 1.8% agarose gel and finally stained with ethidium bromide. A molecular size marker was used as standard marker.

Analysis of RAPD data

RAPD bands were treated as presence or absence, without considering their percentage. For estimating genetic distance among the tested samples; each of DNA bands was treated as a unit character (Williams et al., 1990). Results are recorded (Fig.1),(Table 8).

Results and Discussion

HPLC analysis of the hydrolyzed methanol extract of *S. polygamus* (Table 1) allowed the identification of four phenolic acids and five flavonoid aglycones. Gallic acid was identified as the major phenolic acids (76 mg%). Other major identified phenolic acids are chlorogenic (63 mg%), caffeic acid(2.2 mg %)and ferulic acids(4 mg%).Phenolic acids were evidenced interesting biological activities, e.g. antiviral, antibacterial, anti inflammatory and antioxidant activities (Triantaphyllou et al., 2001;Proestos, et al.,2008).

Table 1: PolyPhenolic constituents identified by HPLC

Rt*	Constituent	%Concentration (W/W)[a] *Leaves of S.polygamus*
2.784	Gallic acid	76
6.519	Chlorogenic acid	63
7.297	Caffeic acid	2.2
8.876	Ferulic acid	4
	Total identified phenolic acids	*145.2*
10.800	Quercetin	14
11.566	Naringenin	12
10.889	Luteolin	1839
11.916	Apigenin	33
11.989	Kaempferol	11
	Total identified flavonoids	*1909*

*R_t=Retention time in minutes

[a]= %Concentration (mg/100gof dry weight

Major identified flavonoid aglycones are luteolin, kaempferol, quercetin, naringenin, and apigenin. Where luteolin being detected as the major flavonoid aglycone(1839 mg/100g dry weight). The amount of phenolic acids reached (145 mg /100 g dry weight) and that of identified flavonoid aglycones (1909 mg/100 g dry weight) of the leaves of *S. polygamus.* To the best of our knowledge, this is the first report on the chemical composition of the leaf extract of *S.polygamus* cultivated in Egypt. The flavonoid contents of the leaves of *S. polygamus* are differ from the previously reported (Erazo et al 2006; Gonzalez et al 2004). The differences may be due to climatic and other extrinsic conditions such as where and when the samples were collected.

The ethanol extract of *S. polygamus* leaf did not cause any mortality up to 10 g/kg b. wt. for 24 hours when given to male albino rats meaning a high margin of safety and encouraging to continue our investigation on the plant, as part of on-going study of medicinal herbs belongs to family Anacardiaceae (Shabana et al.,2008 a, b). Hepatic protection was evidenced by the ability of *S. polygamus* leaf extract at a dose level (100mg/kg.bwt) to decrease the high enzyme parameters by (100%) for AST,(64%)for ALT and(50%) comparable to silymarin at a dose level (25mg/kg.bwt) (Table 2). The presence of phenolic acids and flavonoids, in *S. polygamus* may justify the liver protection since both classes are known to offer such activities (Yang et al., 2010).

Table 2. Effects of the ethanol extract of *S polygamus**and silymarin** drug in male albino rats on serum AST, ALT, and ALP levels.

Group		Zero time	30 days[a]	72 h[b]		30 days[b]	
		Mean±S.E.	Mean±S.E.	Mean±S.E.	% change[c]	Mean±S.E.	% change[D]
AST (u/L)	Control	29.3 ± 0.6	31.1 ± 1.2	142.6 ± 4.9	386.6	151.9 ± 5.5	6.5
	S.polygamus (100 mg/kg b wt)	28.9±0.8	28.1±0.7	68.2±3.1*	192.6	59.2±2.6*•	34
	Silymarin	31.3 ± 1.4	29.2 ± 0.8	43.6 ± 2.5	39.5	28.9 ± 0.8*•	33.7
ALT (u/L)	Control	31.5 ± 1.1	30.9 ± 1.3	162.3 ± 1.9	415.2	171.9 ± 5.2*•	5.9
	S.polygamus (100 mg/kg b wt)	28.1±0.7	28.6±0.5	81.2±2.1*	164.1	66.7±2.5*•	19
	Silymarin	29.7 ± 0.9	29.1 ± 0.6	39.6 ± 1.1	33.3	27.8 ± 0.9*•	29.7
ALP (u/L)	Control	7.3 ± 0.1	7.4 ± 0.1	38.8 ± 1.2	431.5	45.9 ± 2.3*•	18.2
	S.polygamus (100 mg/kg b wt)	7.4±0.1	7.6±0.1	21.2±0.7*	203.0	17.6±0.8*•	17
	Silymarin (25 mg/kg b wt)	7.1 ± 0.1	6.9 ± 0.1	11.2 ± 0.8	57.7	7.4 ± 0.1•	33.9

*statistically significant different from zero time group at p< 0.01; • Statistically significant different from 72h group at p< 0.01; [a] pretreated with tested sample; [b] after induction of liver damage; * Extracts was tested at (100 mg/kg b wt); ** Silymarin at dose level (25mg/kg b wt).
[C] % change (change in liver enzymes after induction of liver disease from zero time)
[D] %change (change in liver enzymes after 30 days from induction of liver disease

Oedema inhibition was calculated to be (80%) of that of indomethacin at the experimental dose level the results recorded (Table 3). This activity could be attributed to presence of polyphenols and triterpenes (Erazo et al., 2006 ;Gonzalez et al., 2004),which have been specifically well recognized for its anti-inflammatory and anti cancer activities(Owoyele et al., 2008; Babu et al.,2001; Saleem, 2009). The demonstrated experimental studies recorded (Tables 3-5) supports the use of *S. polygamus* in folk medicine for the treatment of inflammations, and pain (Erazo et al., 2006). The leaf ethanol extract significantly increased the voltage needed to emit a cry by (78% and 97%) after 1 and 2 hr, respectively meaning significant effect as analgesic agent. The test extract evidenced hypothermic effect at (100mg/kg.bwt) comparable to paracetamol at (50mg/kg.bwt) dose level on hyperthermic rats.

Table3. Effect of the ethanol extract of *S .polygamus* and indomethacin on carrageenan- induced hind paw oedema in male albino rats (n=10).

Group	Dose in mg/kg.b.wt.	% Oedema[a]		Potency [c]
		Mean ± S.E.	% of Oedema inhibition [b]	
Control	1 ml saline	61.8±1.4	-	-
Ethanol extract	100	27.2±0.6*	56	0.8
Indomethacin	20	22.1 ± 0.3*	64.2	1

* Statistically significant different from control group at p < 0.01
[a] % oedema = wt of right paw-wt. of left paw x 100 / wt. of left paw**;** [b] % oedema inhibition (% of change) = $(M_c - M_t) \times 100 / M_c$; M_c is the mean oedema in control rats; M_t is the mean oedema in drug-treated animals; [c] Potency calculated as regard the standard drug.

Table 4 . Analgesic activity of ethanol extract of *S .polygamus* and Novalgin in male albino rats (n=10)

Group	Dose in mg/kg b.wt.	Volts needed before treatment (zero time)	Volts needed after single oral dose					
			One hour			Two hours		
			Mean ± S.E	% of change [a]	P[b]	Mean ± S.E	% of change [a]	P[b]
Control	1 ml saline	75.9± 1.6	76.2± 2.1	24.5	-	76.4± 1.8	24.6	-
Ethanol extract	100	80.1±2.8	126.9±4.5*	78.3	0.7	141.6±5.6*	96.7	0.6
Novalgin	50	79.2± 2.9	169.3± 5.4	134.6	1	182.1± 6.5	150.7	1

* Statistically significant different from zero time at $p < 0.01$; [a] % of change, calculated as regards the effect at zero time; [b]P, potency calculated as regard the standard drug.

Table5. Effect of ethanol extract of *S .polygamus* and paracetamol drug on yeast- induced hyperthermia in male albino rats (n=10).

Group	Dose in mg/kg b.wt.	Induced rise in temp. (°C) before treatment at zero time	Body Temperature change					
			One hour		[b] P	Two hours		[b] P
			Mean± S.E	% of change [a]		Mean ± S.E	% of change [a]	
Control	1 ml Saline	38.4±0.2	38.7±0.1	0.8	-	39.2±0.4	2.1	-
Ethanol extract	100	39.7±0.5	38.3±0.4*	3.5	0.5	37.3±0.1*	6	0.6
Paracetamol	50	39.1±0.5	37.8±0.1*	3.3	1	36.9±0.1*	5.6	1

Significantly different from the corresponding induced rise in temperature of the tested group at P <0.01
[a] % of change, calculated as regard the control group; [b] P, potency calculated as regard the standard drug.

The ethanolic extract of the leaves showed (Table 6) different antimicrobial activities against Gram positive and Gram negative bacteria of variable potencies (43%-57%) comparable to that of Ofloxacin. The phenolic constituents may contribute to these activity significantly (Proestos, et al.,2008;Triantaphyllou, etal.,2001).

On assessing the cytotoxic activity, the leaf ethanol extract of *S. polygamus* showed *in vitro* cytotoxic activity against the tested human carcinoma cell lines especially on human colon carcinoma cell line (IC_{50} 2.62µg/ml) compared to the standard Doxorubicin(IC_{50} 0.4µg/ml) (Table 7)

Concerning the analysis of RAPD data, *S. polygamus* was subjected to RAPD assay of the genomic DNA using five different primers. The banding profile produced by the five random primers for primers: OPA-13, OPA-14, OPA-15, OPB-01, and OPC-20, is illustrated in (Table 8)(Fig.1) RAPD bands were treated as present or absent, without considering their percentage. The number of RAPD-PCR fragments indicated that the five primers were reproduced. Each DNA band was treated as a unit character. A total of 45 different fragments have been recorded, produced mainly by four of the five used primers, showing 11 bands by primer OPC-20 ranging from 1.61 to 0.29 Kbp, 14 bands by primer OPA-13 ranging from 0.85 to 0.16 Kbp, and 10bands by primer OPB-01 ranging from 1.35 to 0.24 Kbp while primers OPA-15 and OPA-14 produced only 8 and 2 bands, respectively. The analysis of RAPD data can thus select the use of primers OPA-13, OPC-20and OPB-01 for the selective discrimination of Egyptian *S.polygamus* from other varieties. These primers may be used as an indicator for obtaining genetic markers.

Table 6 . Results of the antimicrobial testing of the ethanol extract of *S .polygamus*

Microrganisms	Diameter of Zone of inhibition [a]		
	Leaves	Ofloxacin	Fluconazole
Bacillus subtilis ATCC 6051	18 (43%)	42 (100 %)	
Staphylococcus aureus ATCC 12600	16 (43%)	37 (100 %)	--
Streptococcus faecalis ATCC 19433	18 (55%)	33 (100 %)	--
Pseudomonas aeruginosa ATCC 10145	19 (48%)	40 (100%)	--
Escherichia coli ATCC 11775	15 (41%)	37 (100 %)	--
Neisseria gonorrhea ATCC 19424	20 (50%)	40 (100 %)	--
Aspergillus flavus	0.0	--	10 (100 %)
Candida albicans ATCC 26555	16 (57 %)	28 (100 %)	10 (100 %)

a Relative inhibition zone to standard calculated as 100%

Table 7 . Results of cytotoxic activity of the ethanol extract of *S. polygamus*

Extracts	IC_{50} µg/ml		
	Liver carcinoma HEPG2	Larynx carcinoma HEP2	Colon carcinoma HCT116
Ethanol extract of leaves	3.76	5.37	2.62
Standard drug (Doxorubicin)	0.6	0.69	0.4

Conclusion

The anti-inflammatory, analgesic, and antimicrobial activities support scientifically the use of *S. polygamus* in folk medicine for the fore mentioned variable uses. The content of plant constituents as polyphenols of the leaves of *S.polygamus* justify to some extent the cytotoxicity, antibacterial, anti-inflammatory, analgesic activity with pain tolerance. The noticeable hepatoprotective effect demonstrated by the ethanolic extract is mainly attributed to the high contents of flavonoids which exert high potency as free radical quenching properties. The Egyptian plant is differ from *S.polygamus* collected in Argentine and the Chilean species in the flavonoid contents where the Egyptian plant is rich in luteolin where the other varieties rich in quercetin. Also the Egyptian variety shows more broad antimicrobial spectrum against other Gram positive and Gram negative and fungi than that tested on the Argentine and Chilean species. The biological activity evidenced in our study *viz*, hepatoprotective, anti-inflammatory, analgesic, antimicrobial and cytotoxicity (*in vitro*) of this plant cultivated in Egypt are first to be reported in the literature.

Table 8: Molecular size in base pairs of amplified DNA fragments produced by five decamer primers

Molecular size (bp)	A-13	A-14	A-15	B-01	C-20
160	+	-	-	-	-
235	-	-	-	+	-
252	+	-	-	-	-
280	-	-	-	+	-
290	-	-	-	-	+
300	+	-	-	-	-
334	+	-	-	-	-
346	-	-	+	-	-
371	-	-	-	+	+
384	+	-	+	-	+
397	+	-	-	-	-
412	-	-	+	-	-
426	+	-	-	-	-
457	-	-	-	-	+
473	+	-	-	+	+
490	-	+	-	-	-
508	+	-	-	-	-
526	-	-	+	-	-
564	-	-	-	-	+
584	-	-	-	+	-
605	+	-	-	-	+
626	-	+	-	-	-
648	-	-	+	-	-
672	+	-	-	+	-
695	-	-	+	-	-
772	+	-	-	+	-
828	-	-	-	+	-
838	+	-	-	-	-
855	+	-	-	-	-
888	-	-	-	+	+
920	-	-	-	-	-
953	-	-	-	-	+
986	-	-	-	+	-
1058	-	-	+	-	-
1175	-	-	-	-	+
1217	-	-	-	-	-
1351	-	-	-	-	+
1449	-	-	+	-	-
1609	-	-	-	-	-
1851	-	-	-	-	-
SUM	14	2	8	10	11

(+) and (-): presence and absence of band.

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