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Pharmacognostical and biological exploration of *Scaevola taccada* (Gaertn.) Roxb. grown in Egypt

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ABSTRACT

Identification criteria and quality control of *Scaevola taccada* (Gaertn.) Roxb. cultivated in Egypt were scanty. The present study aims to appraise characters of *S.taccada* grown in Egypt, as a traditional medicinal plant. A detailed botanical study of leaf, stem, and flower of *S. taccada* were investigated to find out their characters in entire and powdered form. DNA fingerprinting of leaves was carried out using two polymerase chain reaction (PCR) dependent techniques. Total carbohydrate, lipid and protein content were estimated. Lipoids were subjected to gas liquid chromatography (GLC). Spectrophotometric analysis of polyphenolic contents was conceded out. Comparing the results of the two PCR-dependent techniques revealed that inter-simple sequence repeat (ISSR) will be more useful and informative than random amplified polymorphic DNA (RAPD) in identification of *S.taccada*. Proximate analysis of dried leaf powder showed total, water soluble, acid insoluble ash and moisture content as 14.95%, 5.07%, 5.07% and 9.11w/w respectively. Nutritive value examination revealed a high protein 12% and appreciable carbohydrates contents. *n*-pentacosane 27.70%, stigmasterol 13.16% and α-linolenic acid 12.19% were detected as major lipoids. The antihyperglycemic and the anti-inflammatory activities of the ethanolic and aqueous extracts of the leaves were carried out. Both the ethanolic and the aqueous extracts (200mg/kg) were exhibited noteworthy hypoglycemic effect alike to gliclazide (10 mg/kg). Furthermore, the ethanolic extract was prevailed a parallel decrease in the cholesterol and triglycerides level in streptozotocin (STZ)-Induced Rat Diabetic Model. Moreover, the ethanolic extract (100mg/kg) evidence a significant anti-inflammatory effect 87.17 and 91.88% for 3, 4h treatment respectively compared to indomethacin.

Keywords: *Scaevola taccada,* Goodeniaceae, macromorphology, micromorphology, DNA fingerprint, antihyperglycemic, anti-inflammatory.

1. INTRODUCTION

Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine which will help us to justify its safety and efficacy.(1) Intended for this purpose we have done a pharmacognostical and biological studies of *Scaevola taccada* (Gaertn.) Roxb. family Goodeniaceae grown in Egypt. Goodeniaceae is a family of flowering plants included within the genus Asterales and considered as a sister group to family Asteraceae.(2) It comprises 11 genera and approximately 400 species.(3) Most Goodeniaceae genera are indigenous to Australia, one genus; *Scaevola* is mainly dispersed throughout the pacific area. (4)Members of the family are either herbs or shrubs.(5)The largest genera are *Goodenia*, *Scaevola* and *Damperia.*(6) Family Goodeniaceae is characterized by the presence of some important phytoconstituents: coumarins, (7) iridoid glycosides, (8) pentacyclic triterpenoids: myricadiol and taraxerol,(9) betulin and betulinic acid (10) and inulin.(11) Based on recent researches, these compounds were found to exhibit

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various biological activities of medical importance to treat many diseases.[12]

Scaevola comprises about 130 species, among these 40 are growing outside Australia and two species are widely distributed throughout the Pacific and Indian oceans [4,9] reported that genus *Scaevola* has been used for treatment of diabetes. The name "*Scaevola*" is derived from the Greek word "*Scaevus*" meaning "left-handed[13], also "*Scaevola* " means "little hand."[3] Both names refer to the shape of the flowers which having petals directed to one side giving hand or fan-shaped flowers.

Scaevola taccada (Gaertn.) Roxb. is known as *S. frutescens* (Mill) Krause*, S. plumieri, S. lobelia*[14] and *S.sericea*.[15] It is widely distributed along the coasts of Africa, Indian oceans, tropical Australia and through the pacific oceans from Taiwan to the Hawaiian islands.[4]

Traditionally, different parts of *S.taccada* were used for treatment of various ailments. Leaves were reported to treat indigestion and also used as poultice for headache.[16] The crushed fruits have been used to treat tinea.[15] Medicinally, leaves have been reported to act as anti-diabetic, antipyretic, anti-inflammatory, anticoagulant, skeletal muscle relaxant and also as antimicrobial agent.[17] *S. taccada* is reported to contain phenolic compounds, proteins and carbohydrates, while alkaloids and saponins were completely absent. [18]

DNA-based tools for authentication of medicinal plants is utilized in any form of the drug processed or unprocessed.[19] Random amplified polymorphic DNA (RAPD) has been widely used for the authentication of plant species of medicinal importance. The use of ISSR markers for assessing genetic purity has been reported in agricultural crops like rice, [20] sunflower,[21] maize. [22] By reviewing the current literature, no data was reported concerning the botanical and biological features of *S. taccada* grown in Egypt. Therefore, the main objective was to scrutinize the botanical and DNA profiling as well as corroborate its traditional uses on experimental basis.

Methodology

Plant material

Samples of *S. taccada* (leaves, stems &flowers) used in this study, were collected during the years 2013-2014 from private garden, Giza, Egypt which is specialized in cultivation and spreading of the medicinal plant. The plant material was kindly identified by Agricultural Engineer Therese Labib**.,** Consultant of Plant Taxonomy at Ministry of Agriculture and the Former Director of Orman Botanical Garden, Giza, Egypt. A voucher specimen (28-6-2016) is kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy Cairo University.

Botanical profiling

Specimens of fresh samples of the plant organs under investigation, as well as, samples kept in ethanol (90%) containing glycerin (5%) were used for both morphological and histological studies. Leica light microscope equipped with Leica Queen 550IW digital camera: Leica microsystems, (Wetzlar, Germany) utilized for anatomical examinations. Results were depicted in (Table 1 and Figures1-5).

Table 1: The microscopical measurements of the elements of different organs of *S. taccada*(Gaertn.)Roxb. (Measured in microns)

Element	Length	Width	Diameter	Height
A- The Stem				
Cork	33-50-75	33-37-50		14-21-28
Epidermal cells	35-42-57	28-57-64		8-13-16
Calcium oxalate clusters			9-11-14	
Calcium oxalate prisms	25-35-40	15-20-31		
Pericyclicfibres	305-450-950	8-14-18		

Wood fibres	410-550-750	23-25-38		
Wood parenchyma	85-95-105	22-27-30		
Xylem vessels			11-15-35	
Medullary rays	55-65-75	50-60-65		
Starch granules			8-12-17	
B- The leaf				
Upper epidermal cells	50-60-70	35-45-50		16-20-25
Lower epidermal cells	15-30-60	18-24-42		8-16-25
Neural epidermal cells	24-46-60	19-21-23		9-12-15
Petiole epidermal cells	44-62-106	16-19-22		3-5-6
Stomata	27-30-33	21-24-31		
Calcium oxalate prisms	13-14-18	12-13-14		
Calcium oxalate clusters			6-8-10	
Calcium oxalate rosettes			33-30-42	
Medullary ray cells	60-90-120	39-60-66		
Wood fibers	380-425-470	4-6-11		
Wood parenchyma	165-180-200	30-45-60		
Xylem vessels			12-14-25	
Tracheides	295-318-340	54-63-68		
Starch granules			12-20-23	
Non-glandular trichomes	250-275-287	6-13-19		
C-The flower				
1-Calyx				
Outer epidermal cells	25-35-55	20-25-30		6-9-13
Inner epidermal cells	29-42-57	25-34-40		10-15-20
Stomata	25-30-40	22-25-30		
Non-glandular trichomes	170-175-190	21-23-26		
Xylem vessels			25-29-33	
Tracheids	130-152-161	17-30-35		
Calcium oxalate clusters				7-10-12
2-Corolla				
Outer epidermal cells (apical)	28-40-55	20-28-33		
Outer epidermal cells (middle)	26-28-35	18-23-23		
Outer epidermal cells (basal)	46-60-78	25-30-39		
Inner epidermal cells (apical)	18-45-68	18-31-41		
Inner epidermal cells (middle)	175-190-250	15-30-45		
Inner epidermal cells (basal)	32-41-68	14-23-25		
Stomata	32-34-35	26-27-28		
Xylem vessels			19-22-33	
Non- glandular trichomes	30-33-35	9-18-22		
3- Androecium :				
Filament epidermal cells	143-157-207	21-25-29		

Anther epidermal cells	21-29-33	23-29-32		
Fibrous layer of anther	25-41-62	19-22-25		
Pollen grains			36-60-71	
Xylem vessels			25-29-33	
Calcium oxalate rosette				
4-Gynaecium				
Ovary epidermal cells	23-29-38	23-29-35		1-3-5
Style epidermal cells	32-43-50	12-14-16		
Calcium oxalate rosettes			3-6-7	
Xylem vessels			14-17-18	
Non-glandular trichomes	77-116-125	11-14-16		
5-Pedicel				
Epidermal cells	31-42-65	17-23-30		8-13-17
Non-glandular trichomes	91-95-98	9-10-12		
Prism of calcium oxalate	20-30-35	13-20-29		
Xylem vessels			8-11-27	

Materials for DNA mapping

Buffers: The following buffers were used: *Extraction buffer*: 1.4 M NaCl, 0.1 M Tris (pH 7.5), 20 mM EDTA, 2%(w/v)N-cetyl-N,N,N-tri-methyl ammonium bromide(CTAB), 1% (v/v) β-mercaptoethanol (added immediately before use); *Washing buffer*: 1:76% ethanol, 0.2 M Na-acetate; 2:76% ethanol as washing buffer, 10 mM NH_4 O-acetate, TE-buffer;10 mMtris-HCl(pH 8.0), 1mM EDTA,10x; *Reaction buffer*: 100 mMtris-HCl (pH 8.3), 500 mMKCl, 0.01% (w/v) gelatin, chloroform/ isoamyl alcohol 24:1 (v/v), isopropanol, d NTP, Taq DNA polymerase.

Primers: RAPD & ISSR practice were carried out in triplicates by using genomic DNA with 11 decamer primers for reproducibility of the consequences. Six primers were used for the RAPD analysis with the following sequences: OPA-01: 5′CAG GCC CTT C 3, OPA-07: 5′ GAA AGG GGT G 3′, OPA-10: 5′GTA GAC CCG T 3′, OPB-01: 5′GTT TCG CTC C 3′, OPB-07:5′ GGT GAC GCA G 3′, OPM-01:5′ ACG GCG TAT G 3′. Five primers were used for the ISSR analysis with the following sequences: HB-08: 5′ GAG AGA GAG AGA GG 3′, HB-10: 5′ GAG AGA GAG AGA CC 3′, HB-11: 5′GTG TGT GTG TGT TGT CC 3′, HB-13: 5′GAG GAG GAG GC 3′, HB-14: 5′CTC CTC CTC GC 3.

Molecular weight markers: 100bpladder (New England Biolab Co., UK.)

Equipment: DNA Thermal Cycler, (Perkin Elmer, TA. Warrington, UK) for amplification of DNA, agarose gel electrophoresis tool, for separation of RAPD fragments according to size (Gibco BRL Life Technologies, Paisley, UK) and UV Polaroid camera used for) for visualization of fragments.

Methods for molecular investigations

DNA extraction:

DNA analysis was conducted at Food Technology Research Institute, Agriculture Research center, Ministry of Agriculture and Land Reclamation, Giza, Egypt in 2016. DNA was extracted using cetyl trimethyl ammonium bromide (CTAB) method.[23] Fifty mg of frozen leaf were pulverized in liquid nitrogen, extracted with 0.8 ml CTAB and precipitated with isopropanol.

Assessment of DNA planning:

DNA concentration was determined by diluting the DNA 1:5 in distilled H_2O. The DNA samples were electrophoresed in 1% agarose gel against 10 µg of a DNA size marker. This marker covers a range of concentration between 95 ng and 11 ng. Thus, valuation

of the DNA concentration in a prearranged sample was achieved by comparing the intensity of fluorescence of the unknown DNA band with the dissimilar bands in the DNA size marker.

Magnification of RAPD, ISSR markers:

The PCRs were conceded out using 100 ng of genomic DNA template subsequent a thermal cyclic program. (24)

Thermocyling Profile:

Magnification of PCR was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems, USA) automatic to accomplish 35 cycles later than an early denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 45ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer expansion segment was wholesale to 7 min at 72ºC in the closing cycle. The augmentation products were determined by electrophoresis in a 1.5 % agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 1kb DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (Bio-Rad 2000, Germany).

Data analysis

The banding patterns generated by RAPD, ISSR-PCR marker analyses were scored as present (1) or absent (0), each of which was treated as a sovereign character in spite of its intensity (Fig.6). Only major and reproducible bands obtained for each RAPD, ISSR primer were measured. By comparing the banding patterns of species for a primer, species-specific bands were identified. Faint or indistinct bands were not measured.

Determination of Proximate and Macronutrient Composition

Proximate analysis included the determination of certain analytical standards (ash value and moisture content), as well as that of macronutrient (i.e. total or crude carbohydrate, lipid and protein) contents according to published procedures. (25,26)

Investigation of the Lipoidal Contents

Gas chromatographic profiling of the lipoids

One gram of the petroleum ether fraction of the leaves of *S.taccada* was subjected to saponification. The unsaponifiable and saponifiable lipoids were prepared from the petroleum ether extract (PE) of the leaves of *S. taccada* subjected to gas liquid chromatography (GLC).

Preparation of the unsaponifiable matter

The unsaponifiable matter (USM) was prepared from the petroleum ether extract (PE, 1.0 g) according to Vogel (1975).(27) The solvent-free residue (0.30g), representing the USM, was saved for further GLC analysis. The aqueous alkaline solution, left after separation of the USM, was acidified with dilute hydrochloric acid (5N) to liberate the free fatty acids (FA). These were extracted with diethyl ether (4×50mL). The extract was then dehydrated over anhydrous sodium sulphate and the solvent evaporated to dryness yielding a 0.60g residue representing the free FA.(27)

Preparation of the fatty acid methyl esters (FAME)

The FA mixture as well as the standard fatty acids was, separately, dissolved in small amounts of anhydrous methanol.(28) After 10 min, the solvent was evaporated at room temperature under a stream of nitrogen and the dried residue saved for GLC analysis.

GLC analysis of the unsaponifiable matter (USM)

USM was subjected to GLC on Hewlett-Packard HP-5890 N system equipped with an FID detector,280°C; air flow rate: 350ml/min and H2 flow rate 50ml/min. Analysis was performed on a ThermoTR-5-MS coated with 5% phenyl polysilphenylene siloxane column (30mx0.25mmx0.25µmfilm thickness); injector temperature 270°C, using N_2 as carrier gas and adopting a temperature programming as initial temperature, 70°C,kept isothermal for 2 min, increased to 280°C by the rate of 5°C/min, then kept isothermal. Flow rate 30ml/min. Aliquots,2 µL each, of 2% chloroformic solutions of the USM and reference samples were co-

chromatographed. Identification of the component hydrocarbons, phytosterols and triterpenoids was based on comparison of the retention times observed for the different peaks in the GLC chromatogram of the sample to those of the available authentic samples. The relative amount of each component was calculated *via* peak area measurement using a computing integrator (Table 2).

Table 2: Components identified by GLC analysis of the USM of the leaves of *S. taccada* (Gaertn.) Roxb.

No.	RR_t*	Carbon no.	Identified component	Percentage
1	0.37	C_{14}	n-Tetradecane	1.16
2	0.47	C_{15}	n-Pentadecane	1.59
3	0.49	C_{16}	n-Hexadecane	2.5
4	0.56	C_{17}	n- Heptadecane	2.18
5	0.61	C_{18}	n-Octadecane	13.42
6	0.64	C_{19}	n-Nonadecane	1.93
7	0.72	C_{20}	n-Eicosane	1.29
8	0.76	C_{21}	n-Heneicosane	1.09
9	0.83	C_{22}	n-Docosane	2.66
10	0.87	C_{23}	n-Tricosane	0.73
11	0.95	C_{24}	n-Tetracosane	2.13
12	1	C_{25}	n-Pentacosane	27.70
13	1.06	C_{27}	n-Heptacosane	2.02
14	1.13	C_{29}	n-Nonacosane	3.89
15	1.15	C_{27}	Cholesterol	3.27
16	1.2	C_{28}	Campesterol	2.98
17	1.25	C_{29}	Stigmasterol	13.17
18	1.31	C_{29}	*β*-Sitosterol	6.1
19	1.5	C_{30}	α-Amyrin	2.6
Total identified components				92.41%
Identified hydrocarbons				64.29%
Identified phytosterols				25.52%

*RR_t= Retention time relative to *n*-pentacosane (R_t=22.12 min).

GLC analysis of the fatty acid methyl esters (FAME)

FAME sample was analyzed using GLC Trace GC Ultra system equipped with a FID detector. Analysis was performed using a Thermo TR-FAME column (70%Cyanopropyl Polysilphenylene Siloxane) (30mx 0.25mmx 0.25µm film thickness); injector temperature

200°C, using N_2 as carrier gas and adopting a temperature programming as initial temperature, 140°C, increased to 200°C by the rate of 5°C/min, then kept isothermal for 3min. Flow rate 30ml/min. with N_2 as carrier gas. Aliquots, 2 µL each, of 2% chloroformic solutions of the analyzed FAME and reference fatty acid methyl esters were analyzed under the same conditions. The amounts of individual FA were computed as mentioned under the USM. Identification of the hydrocarbons and sterols was based on comparing the retention time of their peaks with those of the available reference standards. The amount of each component was calculated by peak area measurement using a computing integrator (Table 3).

Table 3: Components identified by GLC analysis of the FAME of the leaves of *S. taccada*(Gaertn.)Roxb.

No.	RR_t*	Carbon no.	Fatty acids corresponding to FAME	Percentage
1	0.55	C_{12}	Lauric acid	0.90
2	0.78	C14	Myristic acid	7.64
4	0.89	C15	Pentadecanoic acid	0.56
5	1	C16	Palmitic acid	34.60
6	1.096	C17	Margaric acid	0.92
7	1.195	C_{18}	Stearic acid	6.19
8	1.22	C(18:1)	Vaccenic acid	7.38
9	1.228	C(18:1)	Oleic acid	0.4
10	1.27	C(18:2)	Linoleic acid (Omega-6)	7.78
11	1.34	$C_{(18:3)}$	Linolenic acid (Omega-3)	12.19
12	1.37	C_{19}	Nonadecanoic acid	6.15
13	1.46	C_{21}	Heneicosanoic acid	0.7
14	1.54	C_{22}	Docosanoic acid	4.55
15	1.62	C_{23}	Tricosanoic acid	1.29
16	1.70	C_{24}	Tetradecanoic acid	1.90
Total identified components				93.15%
Saturated fatty acids				65.4%
Unsaturated fatty acid				27.75%

*RR_t: Relative retention time relative to palmitic acid,(Rt =23.79 min.)

Spectrophotometric determination of phenolic and flavonoid contents

The total phenolic and flavonoid contents were determined in the leaves of *S. taccada* according to published spectrophotometric procedures. [29-31]

Determination of total phenolic content

Spectrophotometric determination of total phenolic content (TPC) was carried out by the Folin-Ciocalteu colorimetric method, as described in the European Pharmacopeia, [30] and modified by Ivanova et al., (2010).[31] The total phenolic content was expressed as Gallic acid equivalents (mg GAE/100mg extract) and

deduced from the pre-established calibration curve. Triplicate experiments were carried out for each sample.

Determination of total flavonoid content

Colorimetric method was adopted, based on measuring the intensity of the color developed when flavonoids are complexes with aluminum chloride method. [29,32] The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per 100 mg extract. Experiments were carried out in triplicates, and average absorbance values recorded.

In-vivo Biological study

Experimental animals

Adult male Wistar rats were obtained from the animal house colony, National Research Center, Giza, Egypt. Animals weighing 150-200 g were used for determination of LD_{50} and evaluation of antihyperglycemic and anti-inflammatory activities. Experimental animals were housed at a temperature of 23 ± 2ºC and 55 ± 5% humidity with 12 hr light/dark cycle, and free access to standard food pellets composed of vitamins mixture (1%), minerals mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein (10.5%) and starch (54.3%). Water was supplied *ad. libitum*. The experimental protocol followed was that of the Institutional Animal Ethical Committee of the National Research Centre.

Determination of Median Lethal Dose (LD_{50})

LD_{50} of the 90% ethanolic extract of the leaves of *S.taccada* was performed in accordance to Organization for Economic Co-operation and Development OECD-425 guidelines.[33] Five Wistar albino rats of uniform weight were selected; one of which was fasted overnight with free access to water. They were given 2000 mg/kg of the tested extract orally and were observed along 24 hrs for mortality. The animal survived and then four additional animals were tested sequentially so that a total of five animals were tested. All the animals were observed closely for 24 hrs daily for 14 days, no mortality was observed. Therefore a dose of 200 mg/kg ($1/10^{th}$ of 2000 mg/kg) was selected as the maximum safety dose. The ethanolic extract was considered safe up to a dose 2 g/kg b.wt.

Evaluation of the antihyperglycemic activity

The ethanolic and the aqueous extracts of the leaves of *S.taccada* were evaluated for their antihyperglycemic activities. Induction of diabetes mellitus was done by a single intraperitoneal (i.p.) injection of streptozotocin (STZ), 55 mg/kg b.wt., freshly prepared in 0.1 M citrate buffer (pH 4.5). [34] A normal control group (n = 6) was injected (i.p.) with the appropriate volume of the citrate buffer. After 48 hrs., blood samples were withdrawn from the retro-orbital venous plexus under light ether anesthesia, and the serum was separated by centrifugation for the determination of the glucose level. The rats having FBG values ≥ 230 mg/dl were selected and considered as hyperglycemic animals and were subjected to further experimentation. Diabetic rats were randomly divided into six groups (six rats each). The first group remained untreated during the whole study period. The second and third groups were administered orally with 100 and 200 mg/kg b.wt. of the ethanolic extract suspension in distilled water, respectively. The fourth and the fifth groups were supplied orally with 100 and 200 mg/kg b.wt. of the aqueous extract and the last group was treated orally with 10 mg/kg b.wt. gliclazide hydrochloride dissolved in distilled water (as standard antihyperglycemic drug). The animals received the indicated treatments every day for 2 weeks. At the end of 2-week treatment, the animals were kept for an overnight fasting and the blood samples were collected from retro-orbital plexus and allowed to clot for 30 min at room temperature. These blood samples were centrifuged at 5000 rpm for 20 min and the serum was obtained for determination of the serum glucose, cholesterol and triglycerides levels.

Determination of glucose level

Blood glucose level was estimated by a test reagent kit according to the method of Trinder, 1969. [35] The absorbance was measured at 510 nm and the results were expressed as mg/dl. Results are recorded in Table (4).

Table 4: Antihyperglycemic activity of the ethanolic and aqueous extracts of *S. taccada* leaves in STZ-induced diabetic rats.

Group	Glucose level (mg/dl)	Cholesterol level (mg/dl)	Triglyceride level (mg/dl)
Normal	86.08±3.76@	115.9±5.68@	106.6±7.76
Control (diabetic)	232.3±14.03*	159.8±11.14	132.0±10.68
Ethanolic extract 100 mg/kg	186.8±10.87* (19.5%)	136.9±5.034 (14.33%)	114.6±3.803 (13.18%)
Ethanolic extract 200 mg/kg	115.3±11.6@ (50.37 %)	120.3±6.671@ (24.71%)	108.0±9.312 (18%)
Aqueous extract 100 mg/kg	185.5±15.68* (20.1%)	141.0±5.341 (11.76%)	131.5±8.825 (0.37%)
Aqueous extract 200 mg/kg	110.2±8.93@ (52.56%)	138.2±6.322 (13.51%)	110.1±7.246 (16.59%)
Gliclazide10 mg/kg	101.7±4.53@ (56.22%)	105.6±9.086@ (33.91%)	106.9±7.846 (19.01%)

*Statistically significant from the normal group at $p<0.05$.

@Statistically significant from the control group at $p<0.05$

Statistical analysis was carried out using repeated one-way ANOVA test followed by Tukey test for multiple comparisons

Determination of serum triglyceride level

Triglycerides level was estimated by enzymatic methods using diagnostic kit according to the method of Fossati and Prencipe, 1982.[36]The absorbance was measured at 510 nm and the results were expressed as mg/dl. Results are presented in Table (4).

Determination of serum cholesterol level

Cholesterol level was estimated by enzymatic methods by using diagnostic kit according to the method of Allain *et al.,* 1974.[37]The absorbance was measured at 500 nm and the results were expressed as mg/dl. Results are recorded in Table (4).

Evaluation of the acute anti-inflammatory activity

The assessment of the acute anti-inflammatory effect of the ethanolic extract of the leaves of *S.taccada* was carried out according to the carrageenan-induced rat paw oedema method. [38]Twenty-four male albino Wistar rats weighing 130-150 g were divided into four groups, each of 6 animals: The first group received 1 ml of normal saline and was considered as (negative control).The second group received the reference indomethacin (20 mg/kg b.wt.) was considered as (positive control).The Third group received 50 mg/kg b.wt. of the plant extract. The fourth group received 100 mg/kg b.wt. of the plant extract. One hour later, oedema was induced by a sub-plantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. The paw volume was measured at 1, 2, 3 and 4 hrafter the induction of inflammation. The difference between initial reading (V_b) and subsequent readings (V_t) gave the change in oedema volume for the corresponding time and calculated according to the following formula: % oedema = $[(V_t - V_b) / V_b] \times 100$

Furthermore, the percentage inhibition of paw oedema in the mean of the treated groups in comparison with the control non-treated group was estimated and calculated according to the following formula:

% Inhibition = [1 - (E_t / E_c)] x 100

Where: E_c = percentage oedema of the control; E_t = percentage oedema of the treated group. The data was presented as mean ± standard error. Results were recorded in Table (5).

Table 5: Anti-inflammatory activity of the ethanolic extract of the leaves of *Scaevola taccada* (Gaertn.) Roxb.

Time (H)	1 hr			2 hr			3 hr			4 hr		
Groups	% Oedema	% Inhibtion	Potency	% Oedema	% Inhibtion	Potency	% Oedema	% Inhibtion	Potency	% Oedema	% Inhibtion	Potency
Control	31.37±0.23	0	-------	33.35±1.58	0	------	36.66±1.2	0		40.33±0.1	0	-------
Indomethacin 20mg/kg b.wt.	20.87±0.1*	33.47	100	13.56±0.36*	59.34	100	10.45±0.3*	71.49	100	8.19±0.3*	79.69	**100**
EtOHext 50 mg/kg b.wt.	27.03±0.5*	13.83	41.32	25.86±1.6*	22.45	37.83	23.54±0.2*	35.78	50.04	21.01±0.4*	47.90	**60.10**
EtOH ext.100 mg/kgb.wt.	22.48±4.4*	28.33	84.64	18.96±0.3*	43.10	72.69	13.81±1.0*	62.32	87.17	10.08 ±1.6	73.22	**91.88**

*Significantly different from the control normal inflamed group at $p < 0.05$. Potency was calculated relative to the standard drug indomethacin.

Statistical analysis

Comparisons were carried out using one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparisons test. The minimal level of significance was identified at $p<0.05$.

Results

Macromorphology

Scaevola taccada (Fig.1A-B) is an evergreen, erect, dense, multi-stemmed shrub that measures about 1.5 to 3 m height; the plant flowers from June to August while the fruits appear at the middle of August till October. Main stem is hard, solid, erect and cylindrical with thick brown cork showing longitudinal fissures, transverse cracks and scattered lenticels. The branching is sympodial. The old branches are hard to break having fibrous fracture and exhibiting pale yellow interior. Young branches are glabrous, woody, cylindrical green in color, showing fine longitudinal striations (Fig.1A). Leaves are petiolate, alternately arranged, crowded at the tip of the stem. Leaf lamina is green in color, simple, obovate in shape measures from 6to 16 cm in length and from 2.5 to 6 cm in width at the widest part. Leaves have asymmetric base, obtuse apex and entire revolute margin. The venation is pinnate reticulate, the veins leave the midrib at angle 55 - 60 ºC and are running towards the margin where they anastmose, the upper surface is dark green and the lower is light green in color. The midrib is more prominent to the lower surface. Both surfaces appeared glabrous except the base showing tuft of hairs. The petiole is very short, flattened, pale green and glabrous. The branches possess a characteristic odour and slight bitter taste (Fig.1B).The inflorescence (Fig. 1B)is bore in group of three in short, axillary, cymose inflorescence. The flowers (Fig.1B); are small, zygomorphic hermaphrodite white sometimes with purple streaks, pedicellate, having short green pedicle measuring0.2-0.7cm long. The flowers have the following floral formula: $K_{(5)}$, $C_{(5)}$, A_5, G_2. Calyx (Fig.1B) is persistant, synsepalous, epigenous formed of 5 cup-shaped united green sepals. Sepals are linear in shape with entire margin and acute apex. The corolla (Fig. 1B) consists of 5 white densely hairy petals united at the base forming a tube is nearly about 3-3.5 cm long. The corolla is splits along one side giving the flowers a distinctive fan-like shape; petals are nearly obovate, pale green to the outside and white to the inside. The androecium (Fig.1B) is epigenous consist of 5 free fertile stamens; which are equal in length with basifixed anthers. The gynaecium (Fig.1B) is consisting of

synocarpous, bicarpellary bilocular, inferior ovary with axile placentation. Style is green incurved at the apex facing the fan-shaped corolla and terminated with bilobed stigma which is surrounded by a cup-shaped like structure fringed with hairs called "indusium"(Fig.1B). The flowers are odourless with no particular taste.

Micromorphology

A transverse section in the old stem (Fig.2A) exhibits a circular outline. It is formed of cork followed by a narrow secondary cortex. The endodermis is indistinct. The pericycle is formed of patches of lignified fibers occasionally interrupted by parenchyma (Fig.2A). The vascular tissue is wide representing about 1/5 of the diameter and formed of a continuous ring of open collateral vascular bundles traversed by biseriate to triseriate medullary rays. The central pith is relatively narrow, constituting about 1/4 of the diameter and containing scattered clusters and prisms of calcium oxalate. The cork (Fig.2A), it is formed of 4-6 rows of radially arranged tangentially elongated cells with thick suberized walls, appearing nearly polygonal isodiametric in surface view. The cortex (Fig. 2A), it is formed of 13-16 rows of slightly tangentially elongated thin walled parenchyma cells containing prisms of calcium oxalate and large rounded starch granules. The pericycle (Fig.2A, C), it is formed of patches of lignified fibers interrupted with thin-walled parenchyma cells. Pericyclic fibers are fusiform, long with moderately wide to narrow lumina, having straight or undulating walls and acute tapering apices.The vascular tissue (Fig. 2A), it is consists of a complete ring of open collateral vascular bundles which traversed by biseriate to triseriate medullary rays. The phloem (Fig.2A) consists of soft phloem tissue formed mainly of thin walled parenchymatous cells, sieve tubes and companion cells. Phloem parenchyma cells contain prisms of calcium oxalate. The xylem (Fig.2 A & C) is lignified and formed of radially arranged elements. Xylem vessels are mostly simple, showing lignified spiral, annular and pitted thickening. Wood fibers are fusiform with lignified walls, wide lumina and acute tapering apices. Wood parenchyma is rectangular in shape with thick pitted lignified walls. Medullary rays are biseriate to trisreiate, formed of rectangular cells having thin walls. The pith (Fig. 2A) is formed of parenchyma cells, first rows beneath the vascular bundle are rounded having pitted lignified walls while the rest of the cells are non-lignified and containing prisms of calcium oxalate.

The structure of the young stem (Fig. 2B) is almost similar to that of the old stem with the following differences: Absence of cork and presence of epidermal cells which are polygonal, slightly axially elongated to isodiametric, having straight anticlinal walls, covered with thick smooth cuticle and devoid of stomata, cortical tissue is formed of thick-walled collenchyma cells followed by parenchyma, containing prism of calcium oxalate and starch granules and the vascular tissue is much narrower while the pith is wider, and formed of rounded thin-walled parenchyma cells.

Powdered stem (Fig. 2C) is greenish brown in colour with characteristic odour and slightly bitter taste. It is characterized by the presence of the following: Fragments of brown, polygonal, suberized cork cells. Moreover, fragments of polygonal axially elongated epidermal cells having straight anticlinal walls and covered with smooth cuticle and devoid of stomata are detected. Fragments of thin walled parenchyma cells containing prisms of calcium oxalate crystals and starch granules which are simple and circular in shape are noticed. Furthermore, fragments of lignified pericyclic fibres with straight or tortuous walls showing moderately wide to narrow lumina and having acute tapering apices are present. In addition to fragments of lignified wood fibres with straight or tortuous walls having wide lumina and acute tapering apices are detected. Xylem vessels with spiral, annular and pitted lignified thickening are present.

A transverse section in the leaf (Fig.3A) shows upper and lower epidermises enclosing in between a homogenous mesophyll. The spongy tissues show

several rows of loosely arranged thin walled parenchyma cells which are loosely arranged. The midrib is more prominent to the lower surface and exhibit three crescent-shaped collateral vascular bundles accompanied by an inverted small one. The first (3-4) rows of cortical tissues are collenchymatous followed by several rows of parenchyma cells containing scattered clusters of calcium oxalate. The vascular system is surrounded with a parenchymatous pericycle. The upper epidermal cells (Fig.3 A,C) are formed of polygonal nearly isodiametric cells with straight thick anticlinal walls, covered with smooth cuticle and devoid of stomata. The lower epidermal cells are formed of polygonal isodiametric to slightly elongated smaller cells with straight anticlinal walls and covered with smooth cuticle. Stomata are of anisocytic type surrounded by three subsidiary cells with few anomocytic. Some epidermal cells show clusters and prisms of calcium oxalate. Trichomes (Fig. 3C) are present at the site of leaf base which areof non-glandular type,they are small, unicellular, unbranched and covered with smooth cuticle. The Mesophyll (Fig.3A) is homogenous undifferentiated. Spongy tissues formed of several rows of irregular shaped parenchyma cells with wide intercellular spaces and containing scattered clusters and prisms of calcium oxalate.Smaller vascular bundles of lateral vein are also present in the region of spongy tissue. Midrib (Fig.3A), consisting mainly of several rows of parenchyma cells with 3-4 rows of subepidermal collenchyma on upper surface and about 5-6 rows of collenchyma cells on the lower one. The parenchyma cell are thin walled mostly rounded in shape, some parenchyma cells containing large starch granules, scattered clusters and prism of calcium oxalate and few tannin cells are also present. The pericycle (Fig.3A), consists of thin walled parenchyma cells.The vascular tissues consists of four vascular bundles, one of them is smaller and inverted. Each one is crescent in shape and of collateral type, formed of xylem towards the upper side and phloem towards the lower one.Xylem vessels (Fig. 3A,C) are spiral and annular lignified thickening which arranged in radial rows. Tracheids (Fig.3C) are few, lignified with annular thickening. Wood fibers **(**Fig. 3C) are fusiform with lignified walls, having moderately wide to narrow lumen and acute apices. Wood parenchyma (Fig. 3C) is formed of rectangular cells having thick pitted and lignified wall. Uniseriate medullary rays (Fig. 3A, C) traverse the xylem and the phloem are formed of large rectangular radials elongated cells having thin walls and containing numerous rosettes of calcium oxalate. A transverse section in the petiole (Fig.3B) is winged in outline and formed of upper epidermis, cortical tissues showing collenchyma followed by several rows of thin walled parenchyma cells containing clusters of calcium oxalate then lower epidermis. The vascular tissues consisting of arc of several collateral vascular bundles extended to the margins of the transverse section. The epidermis (Fig.3C) consists of polygonal axially elongated cells with thick straight anticlinal walls covered with smooth cuticle. Stomata are few and of anisocytic type.

Powdered Leaf (Fig.3C) is green in color, has characteristic odor and bitter taste characterized microscopically by the presence of fragments of the upper epidermis showing polygonal isodiametric cells with thick straight anticlinal wall, covered with smooth cuticle and devoid of stomata. The lower epidermis are polygonal isodiametric to slightly elongated with thin straight anticlinal walls, covered with smooth cuticle and showing anisocytic stomata with few anomocytic. Fragments of epidermis of petiole showing polygonal axially elongated cells, few anisocytic stomata, thick straight anticlinal wall and covered with smooth cuticle are present. Fragments of non-glandular trichomes, unicellular, unbranched covered with smooth cuticle are noticed. Fragments of medullary rays are large rectangular in shape, having thin walls and containing rosettes of calcium oxalate. In addition to, fragments of lignified narrow xylem vessels with spiral, annular and

pitted thickenings. Lignified wood fibers with straight wall, moderately wide to narrow lumen and others with undulating walls and acute apex are present. Fragments of wood parenchyma, rectangular in shape having thick pitted and lignified walls. Lignifiedtracheids of annular thickening and blunt apex scattered clusters, prisms and rosette of calcium oxalate are observed. Starch granules of simple and compound type are present.

A transverse section in the flower at the upper part of the calyx representing sepals, petals, filament and style as shown (Fig.4a) sepals is planoconvex composed of outer and inner epidermises enclosing in between a homogeneous cortical parenchymatous cells, traversed by small collateral vascular bundles. The outer (lower) epidermis (Fig. 5A) consists of polygonal isodiametric cells to slightly elongated with straight anticlinal walls, covered with smooth cuticle, stomata of anomocytic type, trichomes are non-glandular and cells contain prisms and numerous rosettes of calcium oxalate. The inner (upper) epidermis (Fig.5A) consists of large polygonal isodiametric cells, with straight anticlinal walls, covered with smooth cuticle, devoid of stomata and cells contain rosettes and prisms of calcium oxalate. Trichomes (Fig.5A) are present on outer epidermis, they are non-glandular unicellular, curved and covered with smooth cuticle.

A transverse section through the corolla tube (Fig.4e,f)showed five united lobes, each lobe is nearly planoconvex, showing an outer and inner epidermises enclosing in between a homogeneous mesophyll (Fig. 4 e,f) consisting of 14-16 rows of parenchymatous cells having thin cellulosic walls containing scattered prisms and rosettes of calcium oxalate, traversed by small collateral vascular bundles (Fig.4e,f).The outer epidermisin the apical region (Fig.5G)is formed of polygonal nearly isodiametric cells, having straight to slightly wavy anticlinal walls and covered with smooth cuticle, stomata of anomoyctic type and trichomes are of non-glandular type, unicellular and covered with smooth cuticle. Cells contain prisms and numerous rosettes of calcium oxalates. In the middle region (Fig.5H), the outer epidermal cells becoming polygonal isodiametric to slightly elongated cells having straight anticlinal walls, covered with faintly striated cuticle. The outer, inner epidermal cells of the basal region (Fig.5I)are more axillay elongated with straight anticlinal walls. Trichomes are absent. The inner epidermis in the apical region (Fig.5J) is formed of polygonal slightly axially elongated cells having thick straight anticlinal wall.In the middle region (Fig.5K), epidermal cells becoming axially elongated having slightly thick straight anticlinal walls.

A transverse section in the flower at the androecium level (Fig. 4g) showed five stamens of anthers and all were enclosed the five united petals. A transverse section in the anther shows (Fig. 4j) two anther lobes attached by a connective tissue enclosing a vascular bundle. Each anther lobe has two pollen sacs containing numerous reddish brown pollen grains. The epidermis (Fig.5B)consists of polygonal isodiametric cells having thick, wavy anticlinal walls and covered with smooth cuticle. Stomata and trichomes are absent. The fibrous layer (Fig. 5B)is formed of one raw of radially elongated cells which become thickened with lignified bar-like thickenings from the side view and appear with beaded walls from the top view. The pollen grains (Fig.5B) are large spherical in shape; appearing oval from side view, having smooth exine with three germ pores and three germinal furrows. A transverse section in the filament (Fig.4i), consists of an epidermis enclosing a parenchymatous mesophyll, showing scattered rosettes of calcium oxalate, and traversed by one central collateral vascular bundle. The epidermal cells (Fig.5E) are formed of polygonal, axially elongated cells having straight anticlinal walls and covered with smooth cuticle devoid of stomata and trichomes. A transverse section in the ovary (Fig.4 l) appears bilocular and containing one large ovule attached to an axile placentation. The ovary wall consists of an epidermis enclosing parenchymatous

ground tissue, traversed by several vascular bundles (8-10) and shows prisms and numerous rosettes of calcium oxalate. The epidermal cells (Fig.6A) are formed of polygonal isodiametric cells with straight anticlinal walls and smooth cuticle and devoid of stomata. Trichomes of non-glandular type are present.

A transverse section in the style (Fig.4n) is somewhat oval in outline. It consists of an epidermis surrounding a wide parenchymatous ground tissue contains rosette of Ca ox which is traversed by 4-6 small vascular bundles. The epidermal cells (Fig.6A) of the style are polygonal axially elongated with straight anticlinal walls and covered with smooth cuticle. Stomata and trichomes are absent. The stigmatic surface (Fig.6A) shows a papillosed epidermis. The papillae are short having swollen rounded apices and covered with smooth cuticle. The indusium (Fig.6A) is a cup-shaped like structure fringed with long, numerous trichomes. It surrounds the papillosed stigma and consists of polygonal axially elongated cells with straight anticlinal walls, covered with smooth cuticle and containing rosettes of calcium oxalate. Few stomata are present. Trichomes are very abundant on indusium surface, they are non-glandular and unicellular.

A transverse section in the pedicel (Fig.4q) is round in outline. It is formed of epidermis, a cortex and vascular system which is formed of 6-8 collateral vascular bundles arranged in a circle surrounding a parenchymatous area of pith. The epidermis (Fig.6B) consists of polygonal axially elongated cells, having straight anticlinal walls and covered with smooth cuticle. Trichomes are short non- glandular unicellular covered with smooth cuticle.

The powder of the dried flower is pale yellow in color, odorless and tasteless. It is characterized microscopically by the presence of the of the outer, inner epidermis of the sepals formed of polygonal isodiametric cells to slightly elongated with straight anticlinal walls, covered with smooth cuticle, stomata of anomocytic type, trichomes are non-glandular and cells contain prisms and numerous rosettes of calcium oxalate. The inner epidermis of the sepals is devoid of stomata and cells contain rosettes and prisms of calcium oxalate. Outer epidermis of the petal are polygonal nearly isodiametric cells, having straight to slightly wavy anticlinal walls and covered with smooth cuticle, and stomata of anomoyctic type at the apical region The cells at the middle region becoming slightly elongated having straight anticlinal walls and covered with faintly striated cuticle. At the base the cells become more axially elongated. Fragments of the epidermis of the filament, style, indusium and the pedicel consist of polygonal, axially elongated cells having straight anticlinal walls and covered with smooth cuticle and no stomata. The epidermis of anther consists of polygonal isodiametric cells having thick wavy anticlinal walls and covered with smooth cuticle and devoid of stomata. Fragments of fibrous layer of anther showing polygonal elongated cells with lignified bar-like thickenings from the side view and beaded from the top view. Numerous pollen grains which are rounded or oval in shape, having smooth exine, three germ pores and three germinal furrows. The epidermis of ovary consist of polygonal isodiametric cells with straight anticlinal walls and smooth cuticle. Stomata are absent. Lignified xylem vessels having spiral and annular and pitted thickenings (from different parts of the flower) are detected (Fig.5, 6). Numerous scattered rosettes, clusters and prisms of calcium oxalate are presented. Fragments of tracheids having annular thickenings also detected. Several types of non-glandular trichomes short unicellular, having acute and blunt apices and covered with smooth cuticle as well as large unicellular, having blunt apices and covered with warty cuticle are prevailed. Microscopically measurements of the different elements of the flower of *S. taccada* are recorded in Table (1).

Genetic profiling

Unambiguous plant identification is of primary concern to guarantee quality, safety and efficacy of a drug or an extract. Our present study clearly indicated

that RAPD markers could be used effectively to authenticate the plant under investigation in the local herbal markets.

The analysis of ISSR-PCR data can thus select the use of primers HB-11and HB-13 for the selective discrimination of the Egyptian *S.taccada* cultivar from other varieties. These primers may be used as an indicator for obtaining genetic markers. RAPD analysis prevailed of nineteen bands of total different fragments. Which showing 4 bands by primer A-07 ranging from 0.3 Kbp to 0.45 Kbp , 6 bands by primer A-10 ranging from 0.1 Kbp to 0.45K bp , primer B-07 produced 3 bands ranging from 0.3K bp to 0.5 Kbp , and 5 bands are produced by primer M01 ranging from 0.25 Kbp to 0.7 Kbp (Figure 6). Moreover, a total of twenty one different fragments were obtained using ISSR analysis which showing 3 bands by primer HB-08 ranging from 0.2 to 0.3 Kbp, 3 bands by primer HB-10 ranging from 0.3 Kbp to 3.00 Kbp, 7 bands by HB-11 ranging from 0.15 to 0.5 Kbp, HB-13 shows only 4 bands ranging from 0.45 to 3 Kbp and HB-14 shows 4 bands ranging from 0.4 to 0.65 Kbp. HB-11 and A10 primers were produced the largest numbers of bands 7and 6 band with RAPD and ISSR analysis respectively. The least number of bands are produced by HB-08, HB-10, A-07primers (Figure 6). Comparison of the two PCR-dependent techniques revealed that ISSR will be more useful and informative than RAPD in identification of *S. taccada.* Our study showed that there is a large genetic distance between commercial cultivars of *Scaevola* (Purple Fanfare, Pink Perfection, and Mauve Cluster), indicating considerable genetic variation among them. The use of RAPDs in intra- and inter-specific breeding of *Scaevola* is also explored. [39] The genetic diversity of populations of **S. plumieri** within its South African range was examined using Inter Simple Sequence Repeats (ISSR). [40] We have characterized 13 microsatellite loci for **S. taccada**. These microsatellite loci will be useful for estimating population genetic structure possibly resulting from the various seed dispersal patterns of **S. taccada.**[41]

Determination of Proximate and Macronutrient Composition

Proximate analysis of the leaves was carried out to facilitate the detection of the quality and uniformity of the plant where the results showed a total ash (14.95 g%), acid insoluble ash (5.07g%), water soluble ash (5.07 g%), moisture content (9.11 g%) , total protein (12 g%) and carbohydrates (6.55 g%).The analytical standards (total ash, total proteins, total carbohydrates and moisture contents) that are reported here for the first time could serve as useful quality control criteria for conformation of identity and purity of the leaves of the plant. In addition, the leaves could be considered a good source of protein (12%) that is an important building block for muscle, hair and nails. This may explain the traditional use of *S.taccada* leaves for curing skin diseases, in consideration that protein consumption help in building and repairing body tissues. [42]

Investigation of the Lipoidal Contents

GLC analysis of the unsaponifiable matters (USM)

From (Table 2) it could be concluded that: The number of identified components in the USM of the leaves of *S. taccada* was 19 components, representing 92.4%. The hydrocarbons constitute 64.29% of the total composition and detected as a series of alkanes (ranging from C_{14}-C_{29}), where *n*-pentacosane (27.7 %) was the major identified hydrocarbon. The phytosterol content reached 25.52% of the total composition. Stigmasterol was the major identified sterol (13.17%) followed by *β*-Sitosterol (6.13%), cholesterol and campesterol were also detected (3.27, 2.98 %, respectively). The only detected triterpene was α-Amyrin (2.6%). Phytosterols are constituents present in plants that mimic cholesterol. The National Institutes of Health claim that there are over 200 different phytosterols, but the most common plant sterols are: *β*-sitosterol, campesterol, stigmasterol, Δ^5-avenasterol. FDA stated that for foods or beverages containing at least 0.4 g plant sterols, if consumed twice daily (total intake of 0.8 g/day) as a source of diet low in

saturated fat and cholesterol, may decrease the risk of coronary heart disease. The most common natural sources of phytosterols are: almonds, flaxseed, pine nut and sunflower kernels. [43] The high percentage of phytosterols (25.52 %) detected in *S. taccada* leaves may rationalize the significant anti-inflammatory and hypocholesterolemic activities evidenced by the ethanolic extract of the leaves.

GLC analysis of the fatty acid methyl esters (FAME)

Identification of the fatty acids was carried out *via* comparing the retention time of their methyl esters to those of the available reference fatty acids similarly analyzed. From results compiled in Table (3), the following could be concluded: The number of identified fatty acids in the leaves of *S. taccada* (Gaertn.) Roxb. was 16 components representing 93.15%**.**The major identified components were the saturated fatty acids which constitute 65.4% of the total composition of the saponifiable matter, while the unsaturated fatty acids represented only 27.75%. Palmitic acid was the major identified saturated fatty acid (34.60%).Linolenic (9, 12, 15-octadecatrienoic acid) was the predominant unsaturated fatty acid 12.19 % followed by linoleic (omega-6). Omega 3 fatty acids include; alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), while PUFA ω-6 include linoleic acid (LA) and arachidonic acid (AA). Several fatty esters mainly δ-amyrin fatty esters with C_{20}–C_{30} acid moieties were reported from the heartwood and bark of *Scaevola floribunda*. [10] Palmitic acid, oleic acid isolated from *S.spinescens*,while linoleic acid has been isolated from *S.taccada*. Consumption of PUFA ω-3 was found to decrease coronary heart diseases (CHD) occurrence proved by clinical trials through consumption of fish oil or purified PUFA ω-3. PUFA ω-3 not only decreases the total and LDL cholesterol, but also makes small increase in HDL cholesterol. [15]

Determination of total phenolic compounds and total flavonoidal content in the leaves

Spectrophotometric determination of the total phenolic content was carried out using the Folin-Ciocalteau method and expressed as gallic acid equivalent. The total phenolic content of the leaves was 70.59 mg GAE/g dry extract. The total flavonoid content was established by adopting the aluminium chloride colorimetric method and expressed as rutin equivalent. The total flavonoid content of the leaves was found to be 57.12 mg rutin/g dry extract. The majority of bioactive compounds reported in plant materials were phenolic compounds.[44] An optimized extraction condition for the maximum yield of phenolic compounds from *S. taccada* was previously reported to be essential for ongoing assessment of potential biological and anti-cancer activity. [44] Among the tested organic solvents, acetone (78.58 mg GAE/g) exhibited the highest extraction efficiency, followed by methanol and ethanol, which accounted for 89.2 % (70.1 mg GAE/g) and 86.5 % (68.0 mg GAE/g) respectively, of TPC extracted by acetone.[45]Our results prevailed that the TPC evidenced by the Egyptian *S. taccada* is comparable to the reported results by Voung *et al*., (2014). [45]

In- vivo biological activities

Antihyperglycemic effects

Injection of STZ in male Wistar rats resulted in a successful induction of diabetes as indicated by high fasting blood glucose level (FBG) > 230 mg/dl). Also, STZ injection caused an elevation in both cholesterol and triglycerides levels by 37.87% and 23.82% respectively. The calculated data (Table 4) revealed that administration of the ethanolic extract at a dose level of 200 mg/kg b.wt. for 2 weeks, significantly reduces fasting blood glucose (FBG) levels by 50.37% (89.59% potency) when compared to diabetic untreated rats, while the aqueous extract at dose 200 mg/kg b.wt. showed a significant reduction in FBG level by 52.56% (93.49% potency), as compared to gliclazide (10 mg/kg b.wt.)which cause a decrease in FBG by 56.22%.

Moreover, both extracts reduced the measured lipid parameters elevated by induction of diabetes. The ethanolic extract at a dose level 200 mg/kg is more potent

than the aqueous extract as it exhibits a reduction in both cholesterol and triglycerides levels by 24.71% and 18% respectively as compared to standard gliclazide (potency 72.87% and 94.69% respectively). The hypolipidemic effect of the tested extracts is crucial in prevention of diabetes complications. The antidiabetic effect of the ethanolic extract of the leaves of *S.taccada* compared to glibenclamide reported by Umadevi et al.,(2006) [17]was comparable to the establish results in this study. Results are recorded in (Table 6).

Anti-inflammatory activity

The data presented (Table 5) revealed that the ethanolic extract of the leaves at a dose level of 50 and 100 mg/kg b.wt. exhibited a significant anti-inflammatory activity with maximum activity after 4 hours with 60.10% and 91.88% potency, respectively compared to indomethacin (20 mg/kg b.wt.).By reviewing the literature and based on the phytochemical studies of *S. taccada* leaves, the significant anti-inflammatory activity of the ethanolic extract could be attributed to the presence of some phytoconstituents such as terpenes and sterols which are known to have anti-inflammatory action.[46-48]

Discussion

Precise characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine.[1] Intended for this purpose we have done a pharmacognostical and biological studies of *Scaevola taccada* (Gaertn.) Roxb. family Goodeniaceae grown in Egypt. The botanical study of different organs of *S.taccada* are in accordance for the previous reported data. [2,12] Our study showed that there is a large genetic distance between commercial cultivars of *Scaevola* (Purple Fanfare, Pink Perfection, and Mauve Cluster), indicating considerable genetic variation among them. RAPDs in intra- and inter-specific breeding of *Scaevola* is also explored. [39] The genetic diversity of populations of **S. plumieri** within its South African range was examined using Inter Simple Sequence Repeats (ISSR). [40] We have characterized 13 microsatellite loci for **S. taccada**. These microsatellite loci will be useful for estimating population genetic structure possibly resulting from the various seed dispersal patterns of **S. taccada.**[41] The nutritional constitute of the plant may explain the traditional use of *S.taccada* leaves for curing skin diseases, in consideration that protein consumption help in building and repairing body tissues. [42] The most common natural sources of phytosterols are: almonds, flaxseed, pine nut and sunflower kernels. [43] The high percentage of phytosterols (25.52 %) detected in *S. taccada* leaves may rationalize the significant anti-inflammatory and hypocholesterolemic activities evidenced by the ethanolic extract of the leaves. Several fatty esters mainly δ-amyrin fatty esters with C_{20}−C_{30} acid moieties were reported from the heartwood and bark of *Scaevola floribunda*. [10] Palmitic acid, Oleic acid isolated from *S.spinescens*, while linoleic acid has been isolated from *S.taccada*. Consumption of PUFA ω-3 was found to decrease coronary heart diseases (CHD) occurrence proved by clinical trials through consumption of fish oil or purified PUFA ω-3. PUFA ω-3 not only decreases the total and LDL cholesterol, but also makes small increase in HDL cholesterol. [15] Our results prevailed that the TPC evidenced by the Egyptian *S. taccada* is comparable to the reported results by Voung*et al*., (2014). [45] The hypolipidemic effect of the tested extracts is crucial in prevention of diabetes complications. The antidiabetic effect of the ethanolic extract of the leaves of *S.taccada* compared to glibenclamide reported by Umadevi et al.,(2006) [17] and Gheibi et al.,(2017)[46] was comparable to the establish results in this study. By reviewing the literature and based on the phytochemical studies of *S. taccada* leaves, the significant anti-inflammatory activity of the ethanolic extract could be attributed to the presence of some phytoconstituents such as terpenes and sterols which are known to have anti-inflammatory action.[47]

Conclusions

This is the first report on authentication, quality

control and biological evaluation of the plant cultivated in Egypt. Diminutive difference observed between the introduced plant and that grown in Australia, its native area (Nobbs, 2001).[12] Furthermore, the leaves evidenced potential hypoglycemic and anti-inflammatory effects. Phytochemical studies of the aqueous and alcoholic extracts of *S.taccada* leaves are recommended to identify the constituents responsible for those activities.

Conflict of interest

Authors declare no conflict of interest

Author contributions

All authors were contributed in the idea, design the study, draft the article, review the data and edit the article.

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الاستكشاف الدوائي والبيولوجي لـ جودة نبات سكافولا تاكادا(جارتن.) روكسب. المنزرع في مصر

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ملخص

معايير تحديد ومراقبة جودة نبات *سكافولا تاكادا* (جارتن.) روكسب. المنزرع في مصر كانت ضئيلة. لذلك هدفت الدراسة الحالية إلى تقييم سمات سلالة نبات *سكافولا تاكادا* المنزرع في مصر كنبات طبي تقليدي. تم عمل دراسة نباتية مفصلة للاجزاء الكاملة ومسحوق اوراق وساق وزهرة نبات *سكافولا تاكادا* لمعرفة خصائصها. تم إجراء بصمة الحمض النووي للأوراق باستخدام طريقتين تعتمدان على تفاعل البلمرة المتسلسل (PCR). تم تقدير المحتوى الكلي من الكربوهيدرات والدهون والبروتين. تعرضت الدهون للكروماتوجرافيا الغازية السائلة (GLC). تم عمل التحليل الطيفي لمحتويات البوليفينول. كشفت مقارنة نتائج الطريقتين المعتمدتين على PCR أن تكرار التسلسل البيني البسيط (ISSR) سيكون أكثر فائدة وإفادة من DNA متعدد الأشكال المضخم العشوائي (RAPD) في تحديد *سكافولا تاكادا* . أظهر التحليل التقريبي لمسحوق الأوراق المجففة المحتوى الكلي والذوبان في الماء والرماد الغير قابل للذوبان في الحمض ومحتوى الرطوبة 14.95٪ و 5.07٪ و 5.07٪ و 9.11 / وزن على التوالي. أظهر فحص القيمة الغذائية وجود نسبة عالية من البروتين بنسبة 12٪ وايضا محتوى ملحوظ من الكربوهيدرات. وقد اظهرت النتائج ان المحتوى الدهني الرئيسي يشمل n-pentacosane٪27.70 , stigmasterol ٪13.16 و α-linolenic acid 12.19 ٪. تم إجراء الأنشطة الخافضة لنسبة السكر في الدم والمضادة للالتهابات من المستخلصات الإيثانولية والمائية للأوراق. أظهر كل من المستخلصات الإيثانولية والمائية (200 مجم / كجم) تأثير ملحوظ على خفض مستوى سكر الدم على حد سواء بالمقارنة بالجليكلازيد (10 مجم / كجم) ، ساد المستخلص الإيثانولي بانخفاض متوازي في مستوى الكوليسترول والدهون الثلاثية في نموذج الستريتوزوتوسين (STZ) الناجم عن مرض السكري. علاوة على ذلك ، فإن المستخلص الإيثانولي (100 ملجم / كجم) يدل على وجود تأثير مضاد للالتهابات بنسبة 87.17 و 91.88٪ في الساعة الثالثة و الرابعة على التوالي من اعطاؤه للعلاج مقارنة بالإندوميتاسين.

الكلمات المفتاحية: الميكرومورفولوجيا، بصمة الحمض النووي، خافضات سكر الدم، مضاد للالتهابات.

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