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ORIGINAL ARTICLE

Evaluation of antioxidant and anticancer activities of chemical constituents of the *Saururus chinensis* root extracts

Abdullah Alaklabi [a], Ibrahim A. Arif [b], Anis Ahamed [b], Radhakrishnan Surendra Kumar [c], Akbar Idhayadhulla [c,*]

[a] *Faculty of Science, Department of Biological Science, University of Jeddah, Jeddah, Saudi Arabia*
[b] *Prince Sultan Research Chair for Environment and Wildlife, Department of Botany and Microbiology, College of Sciences, King Saud University (KSU), Riyadh, Saudi Arabia*
[c] *PG & Research Department of Chemistry, Nehru Memorial College, Puthanampatti 621007, Tiruchirappalli (Dt), Tamil Nadu, India*

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Abstract Evaluation of antioxidant and anticancer activities were screened by various *Saururus chinensis* root extracts. Four solvents (ethyl acetate, methanol, ethanol, and water) extracts were investigated for their total flavonoids, phenol contents and their antioxidant activity of DPPH (2,2-diphenyl-1-picrylhydrazyl), NO (nitric oxide), H_2O_2 (hydrogen peroxide), ABTS 2,2′-azino-bi s(3-ethylbenzothiazoline-6-sulfonicacid)diammonium assays, FRAP (ferric reducing ability of plasma) assays and anticancer activity. The total phenolic and flavonoid content of extracts were determined by using FC (Folin–Ciocalteu) and $AlCl_3$ colorimetric assay method. Total flavonoid content in these plants ranged from 24.7 to 72.1 mg g^{-1} and amount of free phenolic compounds was between 11.2 and 67.1 mg g^{-1} extract. The all extracts have significant levels of phenolics and flavonoids content. Anticancer activity was screened for MCF-7 breast cancer cell line. Ethanol extract shows significant of antioxidant activity and water extract shows significant of anticancer activity compared with standard (BHT) butylated hydroxy toluene. These ethanol and water extracts could be considered as a natural source for using antioxidant, and anticancer agents compared to commercial available synthetic drugs.

* Corresponding author at: Research Department of Chemistry, Nehru Memorial College (Affiliated to Bharathidasan University), Puthanampatti 621007, Tiruchirappalli District, Tamil Nadu, India.
E-mail addresses: idhayadhulla@nmc.ac.in, a.idhayadhulla@gmail.com (A. Idhayadhulla).
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1. Introduction

Saururus chinensis has been used as a diuretic and a detoxifying agent for edema, jaundice, gonorrhoea and several inflammatory diseases treatment in China and India (Rao and Rao, 1990). More than 4000 different naturally occurring flavonoids were identified from other plant materials (Middleton et al., 1986) but very important pharmacological studies have demonstrated that major flavonoids (Kang et al., 2005; Sung et al., 1997) and other ligands (Hwang et al., 2003a; Sung and Kim, 2000) from *S. chinensis* (Fig. 1), which possess a wide array of pharmacological and biochemical activities, such as antidiabetic (Moon et al., 2008), anti-carcinogenic (Omer et al., 2005; Hester et al., 2003), anti-inflammatory (Hwang et al., 2003b), antioxidant (Lee et al., 2003) and hepato-protective (Kim et al., 2004), neuro-protective activity (Nortier and Vanherweghem, 2002), nephrotoxic (Cheng et al., 2006), carcinogenic (Kohara et al., 2002), and mutagenic (Balachandran et al., 2005). Importantly *S. chinensis* baill shows strong antioxidant activity (Lee et al., 2004; Choi et al., 2002), and its extract has been shown to reduce lipid peroxide levels in rats fed a high-fat diet (Yu et al., 2008). Current cancer chemotherapy is primarily dependent on repeated administrations of synthetic anticancer agents. Although effective, their repeated use has often resulted in the development of resistance, plants have been suggested as an alternative source of materials for cancer therapy because they constitute a potential source of bioactive chemicals. Previous pharmacological studies on *S. chinensis have* reported that its extracts have cytotoxic activities and EtOH extract from the aerial parts of *S. chinensis* showed a potent anti-proliferative activity against C33a human cervical cancer cells (Kim et al., 2011).

Therefore, studies on herbs have become the top issue at present time for their great potential of biological activity, present investigation the estimated total phenolics and flavonoids in dried root of *S. chinensis* in various solvent extracts (methanol, ethanol, ethyl acetate, and water) and screened for antioxidant and anticancer activities.

2. Materials and methods

2.1. Preparation of solvent extract

S. chinensis root powder (100 g) was immersed in 500 mL of double distil water. The filtrate was constant stirring with maximum 72 h. The extract was then concentrated under reduced pressure at 40 °C using vacuum rotary evaporator, the yield of water extract was 15.25 g. The aqueous layer further fractionated with ethyl acetate, methanol, and ethanol fractions were concentrated with vacuum rotary evaporator, yield of each fractions 16.1%, 24.6% and 18.0% respectively.

2.2. Determination of total phenolic and flavonoid content

The total phenolic and total flavonoid were identified by using the FC (Folin–Ciocalteu) and aluminium chloride colorimetric assay methods, the method was followed by previously report method (Marinova et al., 2005). Total phenolic and flavonoid content rang are represented in Table 1.

2.3. Antioxidant activity of DPPH method

DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity was screened for *S. chinensis* root extracts and the method was followed by previously report method (Cefarelli et al., 2006). Accordingly, extracts were examined for their ability to prevent the bleaching of the purple colored methanol solution DPPH 1.6 mL of test sample (25, 50, and 100 μg/mL) and 1.6 mL of 0.004% (w/v) methanol solution of DPPH were reacted at room temperature, absorbance of reaction mixture was measured at 517 nm.

Percentage inhibition was calculated by.

$$\text{DPPH Scavenging effect } (\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

2.4. H_2O_2 scavenging activity

Hydrogen peroxide scavenging activity was screened for various solvent of *S. chinensis* root extracts, the method was followed by previously reported literature method (Ruch et al., 1989). A solution of H_2O_2 (0.6 mL, 40 mM) with phosphate buffer (pH 7.4) and root extract (3.4 mL) with phosphate buffer were reacted, the reaction mixture was measured by absorbance at 230 nm.

Percentage inhibition of H_2O_2 was calculated by.

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100]$$

2.5. NO scavenging activity

Nitric oxide scavenging activity was calculated using by slight modified version of the method devised by Marcocci et al. (1994). Sodium nitroprusside (1 mL, 10 mM) and 1.5 mL of

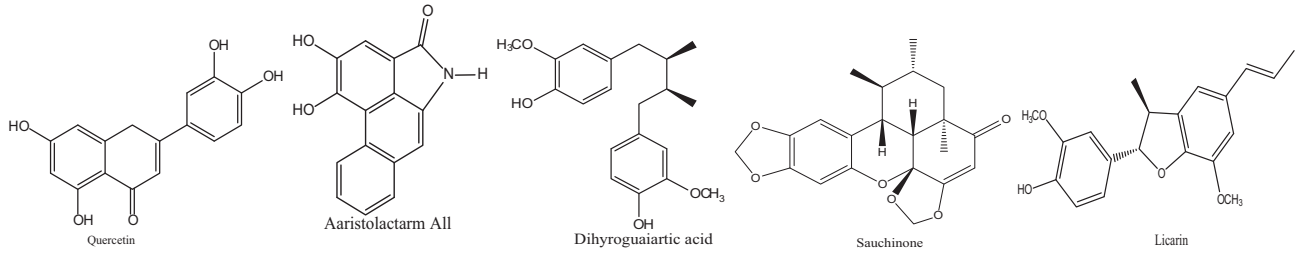

Figure 1 Structure of reference compounds from *Saururus chinensis* leaves extract.

Table 1 The total phenolic and flavonoid content of *Saururus chinensis* root extract.

Extracts	Total phenolic[a] (mg GAE/100 g of dry mass)	Total flavonoids[a] (mg CE/100 g of dry mass)
Ethyl acetate	11.2 ± 0.2	24.7 ± 0.2
Methanol	22.0 ± 0.6	54.0 ± 0.7
Ethanol	35.2 ± 1.2	58.4 ± 0.4
Water	67.1 ± 0.9	72.1 ± 0.1

GAE: Gallic Acid Equivalents; CE: Catechin Equivalents.
[a] Values expressed as mean ± standard deviation ($n = 3$).

phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations of test sample incubated for 150 min at 25 °C. Aliquots (1 mL) of reaction mixtures were then react with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride).

Percentage inhibition of nitric oxide scavenging was calculated by.

$$\% \text{ NO Scavenging} = [(A_{control} - A_{sample}/A_{control}) \times 100]$$

2.6. ABTS radical cation decolourisation assay

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)di ammonium) assay can be used to determine the antioxidant activity of biological fluids, cells, tissues, natural and other synthetic therapeutic compounds. The assay measures ABTS radical cation formation induced by metmyoglobin and hydrogen peroxide. A water soluble trolox is used as a positive control for inhibiting the formation of the radical cation in the assay (Adedapo et al., 2009).

Preparation: The ABTS reagent was prepared by 5 mL of 14 mM ABTS and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$), the reaction mixture was kept in dark room at room temperature for 16 h. The reagent absorbance was adjusted to 0.700 ± 0.02 at 734 nm with distilled water and used for the assay purposes.

1 mL of ABTS reagent is added to different concentrations of test sample measured by 734 nm at 3 min interval.

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

A_0 – absorbance of control, A_1 – absorbance of the tested sample.

2.7. Ferric reducing antioxidant power (FRAP) assay

The FRAP (ferric reducing ability of plasma) assay was performed according to the previous reported method (Dehghan and Khoshkam, 2012). FRAP reagent was prepared by mixing of 2.5 mL of solutions TPTZ (10 mM, (40 mM) HCl, and $FeCl_3$ (20 mM) in 25 mL of acetate buffer (300 mM, pH 3.6), the light blue reagent contains Fe^{3+}-TPTZ that changes to Fe^{2+}-TPTZ as dark blue. These changes were due to the absorbance increase as monitored at a wavelength of 593 nm for different concentrations of *S. chinensis* root extracts in FRAP reagent .

2.8. Anticancer activity

Anticancer assays were performed according to the US NCI protocol, which was described elsewhere (Boyd and Paull, 1995).

Cell viability test: The viability of cells was assessed by MTT assay using MCF-7 cell lines.

Cytotoxic assay (MTT method): The anticancer activity was carried out according to the method described in previous literature (Mostafa et al., 2016).

MCF-7 cell lines was treated with these compounds at one primary cytotoxic assay dose of 100 μM for 48 h (MTT anticancer assay). Doxorubicin was used as a standard. In the current protocol, all cell lines were pre-incubated on a microtiter plate. The results of each test were reported as the growth percentage of treated cells compared to untreated control cells. A 0.1 mL aliquot of the cell suspension (5×10^6 cells/100 μL) and 0.1 mL of the test solution (6.25–100 μg) were added to the wells, with the plates kept in an incubator (5% CO_2) at 37 °C for 72 h. After 72 h, 20 μL of MTT was added, and the plates were kept in the CO_2 incubator for 2 h, followed by the addition of propanol (100 μL). The plates were covered with aluminum foil to protect them from light and subsequently agitated in a rotary shaker for 10–20 min, afterwards the 27-well plates were processed on an ELISA reader to obtain absorption data at 562 nm.

2.9. Statistical analysis

All experiments were performed in triplicate and all data were expressed at least 3 independent evaluations and the standard deviations (SD) were also calculated using Microsoft Excel 2007 software (Microsoft, Redmond, WA, USA).

3. Result and discussion

3.1. Antioxidant activity of S. chinensis root extracts

The *S. chinensis* root extracts were screened for antioxidant property of 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), nitric oxide (NO), ABTS and FRAP assays method.

The antioxidant activity of extracts was measured by radical scavenging ability through UV–visible spectrophotometer. Methanolic solutions of DPPH shows a strong purple color, with strong absorption at 517 nm, and when reduced are a yellow color. Free radical scavenging activity of DPPH radical was found to increase with increase in concentration, showing a maximum of 88.71 at 100 μg/mL. The ethanol extract showed a good capacity of scavenging the DPPH free radical activity and highly active (IC_{50}:11.04 μg/mL) compared with standard BHT at concentration 100 μg/mL. Results of DPPH reduction is shown in Table 2.

The Fig. 2 shows that kinetic activities of different extracts and estimated the scavenging activity of maximum reaction time. The higher antioxidant activity of the extract may be assumed that –OH groups are primarily involved in H-atom transfer reactions to DPPH, which attributed to the highly significant contribution of 3′,4′-hydroxyl groups on the ring.

Table 2 DPPH scavenging activity (%) of *Saururus chinensis* root extracts.

Extracts	Concentration (μg/mL)[a]			IC_{50} (μg/mL)[a]
	25 μg/mL	50 μg/mL	100 μg/mL	
Ethyl acetate	10.31 ± 0.17	23.23 ± 0.14	43.01 ± 0.32	> 100
Methanol	60.62 ± 0.23	71.01 ± 0.16	88.71 ± 0.12	15.13 ± 0.23
Ethanol	69.22 ± 0.19	74.12 ± 0.22	91.02 ± 0.21	11.04 ± 0.31
Water	06.10 ± 0.07	12.03 ± 0.21	21.21 ± 0.11	> 100
BHT	54.21 ± 0.22	70.30 ± 0.12	82.31 ± 0.30	18.86 ± 0.21

[a] Value expressed are means ± SD.

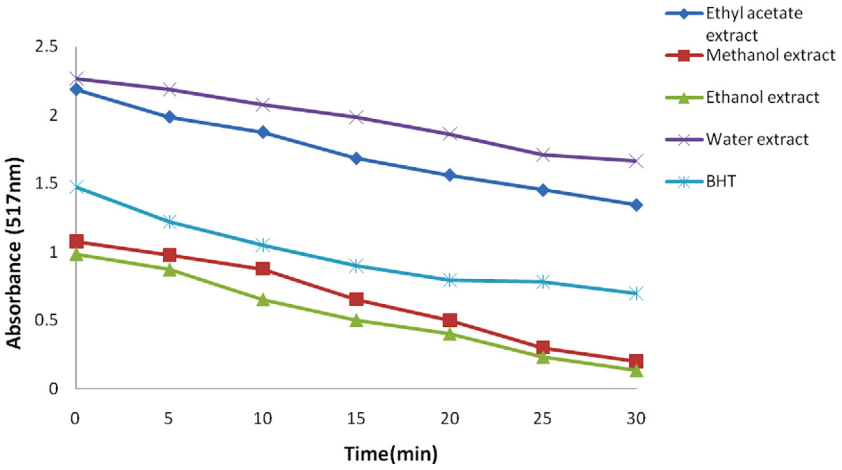

Figure 2 Measurement of absorbance between *Saururus chinensis* root extracts and DPPH solution in kinetic behavior at concentration 100 μg/mL.

Hydrogen peroxide (H_2O_2) scavenging activity showed that ethanol extract (12.00 μg/mL) very effective scavenging activity compared with standard BHT (20.35 μg/mL). Results of H_2O_2 scavenging reduction is shown in Table 3.

The extract showed nitric oxide scavenging activity between 25 and 100 μg/mL concentration. Ethanol extract shows highly active (89.11%) compared with standard BHT (83.32 %) at concentration 100 μg/mL. The nitric oxide (NO˙) radical reacts with Griess reagent, to form formazon which was measured spectrophotometrically. The IC_{50} value of ethanol extract was found to be 14.58 μg/mL. Results of NO scavenging reduction is shown in Table 4. Extracts were screened for ABTS radical-scavenging activity, the EtOH extract (IC_{50}:17.54 μg/mL) was significant of activity compared with standard BHT. Results of ABTS scavenging reduction is shown in Table 5.

The FRAP assay was measured by ability to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+} - (TPTZ)_2]^{3+}$ to provided blue colored ferrous complex $[Fe^{2+} - (TPTZ)_2]^{2+}$ in acidic medium at 593 nm absorbance . The ethanol extract was absorbed very low reducing abilities at 25–100 μg/mL compared with standard BHT, activity variation shows in Fig. 3.

3.2. Anticancer activity of S. chinensis root extracts

The compounds were for primary anticancer assay towards MCF-7 cell lines (human breast cancer). Results were reported as the percent growth of the treated cells when compared to the untreated control cells.

All the synthesized compounds methanol, ethanol, ethyl acetate, and water were evaluated for their anticancer activity

Table 3 Hydrogen peroxide (H_2O_2) scavenging activity of *Saururus chinensis* root extracts.

Extracts	Concentration (μg/mL)[a]			IC_{50} (μg/mL)[a]
	25	50	100	
Ethyl acetate	12.16 ± 0.14	20.21 ± 0.02	43.13 ± 0.04	> 100
Methanol	55.28 ± 0.11	72.00 ± 0.11	84.21 ± 0.16	18.13 ± 0.02
Ethanol	68.47 ± 0.04	84.17 ± 0.09	92.12 ± 0.41	12.00 ± 0.15
Water	16.29 ± 0.35	23.12 ± 0.11	36.12 ± 0.18	> 100
BHT	59.01 ± 0.04	68.51 ± 0.09	82.17 ± 0.53	20.35 ± 0.13

[a] Value expressed are means ± SD.

Table 4 NO scavenging activity of *Saururus chinensis* root extracts.

Extracts	Concentration (μg/mL)[a]			IC_{50} (μg/mL)[a]
	25	50	100	
Ethyl acetate	–	09.14 ± 0.24	14.32 ± 0.41	> 100
Methanol	52.51 ± 0.21	69.16 ± 0.10	82.12 ± 0.11	20.19 ± 0.19
Ethanol	60.01 ± 0.03	71.12 ± 0.64	89.11 ± 0.12	14.58 ± 0.11
Water	0.30 ± 0.05	17.01 ± 0.11	30.10 ± 0.10	> 100
BHT	53.16 ± 0.02	67.65 ± 0.63	83.32 ± 0.07	22.13 ± 0.23

[a] Value expressed are means ± SD.

Table 5 ABTS radical-scavenging activity of *Saururus chinensis* root extracts.

Extracts	Concentration (μg/ml)[a]			IC_{50} (μg/ml)[a]
	25	50	100	
Ethyl acetate	21.30 ± 0.04	35.72 ± 0.11	40.31 ± 0.41	> 100
Methanol	55.23 ± 0.43	62.12 ± 0.10	73.20 ± 0.34	21.32 ± 0.18
Ethanol	59.34 ± 0.24	69.71 ± 0.08	83.23 ± 0.91	17.54 ± 0.32
Water	06.85 ± 0.14	11.32 ± 0.14	19.07 ± 0.12	> 100
Trolox	62.02 ± 0.02	74.09 ± 0.21	92.11 ± 0.56	14.21 ± 0.24

[a] Value expressed are means ± SD.

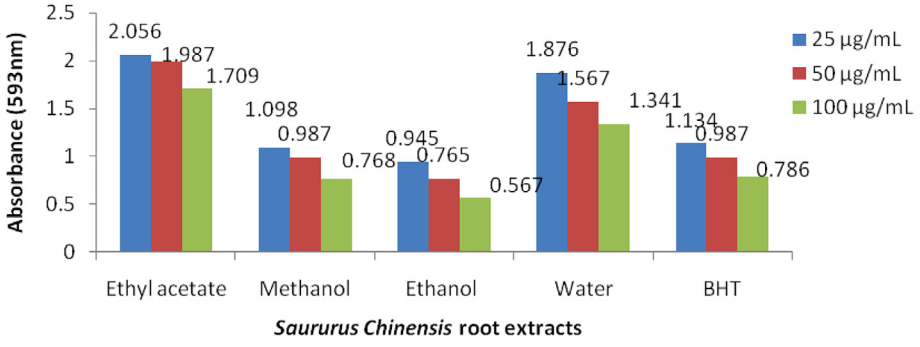

Figure 3 FRAP activity of *Saururus chinensis* root extracts at concentration 100 μg/mL.

Table 6 Anticancer activity of *Saururus chinensis* root extracts against MCF-7 cancer cell line.

Extracts	% Inhibition of cell viability			LD_{50} μg/mL
	25 μg/mL	50 μg/mL	100 μg/mL	
Ethyl acetate	–	–	07.5	> 100
Methanol	–	04.6	18.1	> 100
Ethanol	–	09.4	20.9	> 100
Water	21.6	32.5	54.3	91.2
Standard	52.2	65.8	84.2	24.2

1,3-Bis(4-chlorobenzenesulfonyl)imidazolidine-2-one used as a standard.

against a human cancer cell line such as MCF-7 (Human breast cancer), by employing MTT assay. The results of cell viability data are summarized in Table 6.

The inhibitory concentration (LD_{50}) value was calculated by the test sample of concentration at which 50% of cells viable, and calculated from the logarithmic trend line of the cytotoxicity graphs. The *in vitro* screening results revealed that the compound water exhibited promising anticancer activity compared in MCF7 breast cancer cells (54.2% inhibition) were observed at concentration (100 μg/mL). This inhibition at the mentioned concentration indicates a greater potency of compound water with a strong lethal effect over the breast cancer (MCF-7) cell line computed with other solvent extract.

Compounds methanol, ethanol, and ethyl acetate have low inhibitory activity against MCF-7 cell line.

4. Conclusion

In conclusion, the result of this study demonstrates that four different *S. chinensis* root extracts in methanol, ethanol, ethyl acetate, and water were screened for antioxidant, anticancer activates. An important finding in this study, the *S. chinensis* root ethanol extract was most important in antioxidant activity and water extract is highly active against anticancer activity against MCF-7 cancer cell line. Current investigation of antioxidant, and anticancer activates were evaluated using *in vitro* experiments only and further investigation for mechanism of action and *in vivo* experiments will lead to a more complete assessment of *S. chinensis* root extracts.

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References

Adedapo, A.A., Jimoh, F.O., Koduru, S., Masika, P.J., Afolayan, A. J., 2009. Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of Buddleja saligna. BMC Complement Altern. Med. 9 (21), 1–8.

Balachandran, P., Wei, F., Lin, R.C., Khan, I.A., Pasco, D.S., 2005. Structure activity relationships of aristolochic acid analogues: toxicity in cultured renal epithelial cells. Kidney Int. 67, 1797–1805.

Boyd, M.R., Paull, K.D., 1995. Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. Drug Dev. Res. Drug 34, 91–109.

Cheng, C.L., Chen, K.J., Shih, P.H., Lu, L.Y., Hung, C.F., Lin, W.C., Gu, J.Y., 2006. Chronic renal failure rats are highly sensitive to aristolochic acids, which are nephrotoxic and carcinogenic agents. Cancer Lett. 232, 236–240.

Choi, C.W., Kim, S.C., Hwang, S.S., Choi, B.K., Ahn, H.J., Lee, M. Y., Park, S.H., Kim, S.K., 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163, 1161–1168.

Cefarelli, G., D'Abrosca, B., Fiorentino, A., Izzo, A., Mastellone, C., Pacifico, S., Piscopo, V., 2006. Free-radical-scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. J. Agric. Food Chem. 54, 803–809.

Dehghan, G., Khoshkam, Z., 2012. Tin(II)-quercetin complex: synthesis, spectral characterization and antioxidant activity. Food Chem. 131, 422–426.

Hwang, B.Y., Lee, J.H., Nam, J.B., Hong, Y.S., Lee, J.J., 2003. Lignans from *Saururus chinensis* inhibiting the transcription factor NF-κB. Photochemistry 64, 765–771.

Hwang, B.Y., Lee, J.H., Jung, H.S., Kim, K.S., Nam, J.B., Hong, Y. S., Paik, S.G., Lee, J.J., 2003. Sauchinone, a lignan from *Saururus chinensis*, suppresses iNOS expression through the inhibition of transactivation activity of RelA of NF-kappaB. Planta Med. 69, 1096–1101.

Hester, V.D.W., Anna, G.S., Karin, S., 2003. Biphasic modulation of cell proliferation by quercetin at concentrations physiologically relevant in humans. Cancer Lett. 1, 41–47.

Kang, T.H., Cho, H., Oh, H., Sohn, D.H., Kim, Y.C., 2005. Flavonol glycosides with free radical-scavenging activity of *Saururus chinensis*. Fitoterapia 76, 115–117.

Kim, S.R., Sung, S.H., Kang, S.Y., Koo, K.A., Kim, S.H., Ma, C.J., Lee, H.S., Park, M.J., Kim, Y.C., 2004. Aristolactam BII of *Saururus chinensis* attenuates glutamate-induced neurotoxicity in rat cortical cultures probably by inhibiting nitric oxide production. Planta Med. 70, 391–396.

Kim, H.Y., Choi, T.W., Kim, H.J., Kim, S.M., Park, K.R., Jang, H.J., Lee, E.H., Kim, C.Y., Jung, S.H., Shim, B.S., Ahn, K.S., 2011. A methylene chloride fraction of *Saururus chinensis* induces apoptosis through the activation of caspase-3 in prostate and breast cancer cells. Phytomedicine 18, 567–574.

Kohara, A., Suzuki, T., Honma, M., Ohwada, T., Hayashi, M., 2002. Mutagenicity of aristolochic acid in the lambda/lacZ transgenic mouse (MutaMouse). Mutat. Res. 515, 63–67.

Lee, A.K., Sung, S.H., Kim, Y.C., 2003. Inhibition of lipopolysaccharide-inducible nitric oxide synthase, NF-alpha and COX-2 expression by sauchinone effects on I-kappaBalpha phosphorylation, C/EBP and AP-1 activation. Br. J. Pharmacol. 139, 11–20.

Lee, W.S., Baek, Y.I., Kim, J.R., Cho, K.H., Sok, D.E., Jeong, T.S., 2004. Antioxidant activities of a new lignan and a neolignan from *Saururus chinensis*. Bioorg. Med. Chem. Lett. 14, 5623–5628.

Marinova, D., Ribarova, F., Atanassova, M., 2005. Total phenolic and total flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metall. 40, 255–260.

Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T., Packer, L., 1994. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem. Biophys. Res. Commun. 201, 748–755.

Middleton, E., Kandaswami, C., Harborne, J.B., 1986. The Flavonoids: Advances Research Since. Chapman and Hall, London, UK. 1993.

Mostafa, A.A., Al-Rahmah, A.N., Surendra Kumar, R., Manilal, A., Idhayadhulla, A., 2016. Biological evaluation of some imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one derivatives as anticoagulant agents and inhibition of MCF-7 breast cancer cell line. Int. J. Pharm. 12, 290–303.

Moon, T.C., Seo, C.S., Haa, K., Kim, J.C., Hwang, N.K., Hong, T.G., Kim, J.H., Kim, D.H., Son, J.K., Chang, H.W., 2008. Mesodihydroguaiaretic acid isolated from Saururuschinensis inhibits cyclooxygenase-2 and 5-lipoxygenase in mouse bone marrow-derived mast cells. Arch. Pharm. Res. 31, 606–610.

Nortier, J.L., Vanherweghem, J.L., 2002. Renal interstitial fibrosis and urothelial carcinoma associated with the use of a Chinese herb (Aristolochiafangchi). Toxicology 577, 181–182.

Omer, C., Mehmet, K., Ahmet Sukru, O., 2005. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta cell damage in rat pancreas. Pharmacol. Res. 51, 117–123.

Rao, K.V., Rao, N.S., 1990. Chemistry of *Saururus cernuus*, VI: three new neolignans. J. Nat. Prod. 53, 212–215.

Ruch, R.J., Cheng, S., Klaunig, J.E., 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10, 1003–1008.

Sung, S.H., Kim, Y.C., 2000. Hepatoprotective diastereomeric lignans from *Saururus chinensis* herbs. J. Nat. Prod. 63, 1019–1021.

Sung, S.H., Kwon, S.H., Cho, N.J., Kim, Y.C., 1997. Hepatoprotective flavonol glycosides of *Saururus chinensis* herbs. Phytother. Res. 11, 500–503.

Yu, M.H., Im, H.G., Lee, J.W., Bo, M.H., Kim, H.J., Kim, S.K., Chung, S.K., Lee, I.S., 2008. Effects of ethanol extract from *Saururus chinensis* (Bour.) Baill on lipid and antioxidant metabolisms in rats fed a high-fat diet. Nat. Prod. Res. 22, 275–283.