JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Evaluation of anti-inflammatory and antioxidant activities from Strychnos icaja Baillon (Loganiaceae)

R.R.R. AWORET SAMSENY [1]*, L.E. MENGOME[2], S. ABOUGHE ANGONE[3]

[1,2,3] Pharmacopeia and Traditional Medicine Institute: National Scientific Research and Technology Center, Libreville, Gabon
Email: awsams@yahoo.fr[1], linemengome@gmail.com[2], sophie.aboughe@gmail.com[3]
**Corresponding Author*

ABSTRACT

Anti-inflammatory agents have gained an increasing demand with the rising number of indications of inflammatory mediated diseases. Medicinal plants extract treats numerous diseases mediated by inflammation and remain a potent source of new anti-inflammatory agents and antioxidants. Strychnos icaja Baillon (Loganiaceae) is a tropical shrub common of Central Africa forest. Traditional usage of the Shrub in Gabon, involve haemorrhoids, ear infections, rheumatism and diabetes treatments. The objective of the present study is to analyse the different root's extract phenolic and alkaloids content, along with the evaluation of its in vitro anti-inflammatory and antioxidants activities. Phytochemical revealed the presence of alkaloids in the roots (0.660%). St (Strychnos) dichloromethane type extract highlights polyphenol content (total phenolic: 54.21 ± 4.87 mgEAG/g; total flavonoids: 6.98 ± 1.27 mgEQ/g; total tannins 46.42 ± 1.03 mg EAT/g). St aqueous extract has a very low polyphenol content with (22.28 ± 0.50 mgEAG/g; 1.29 ± 0.45 mgEQ/g and 17.30 ± 0.69 mg EAT/g) according to the above arrangement. Anti-inflammatory activities by inhibition of lipoxygenase and xanthine oxidase are also significant: St total alkaloids roots 94.33 ± 0.16 % vs 77.74 ± 0.14 % respectively; St dichloromethane 86.61±2.83% vs 65.19±5.25 %. Inhibition of both enzymes by St aqueous extract is deficient. DPPH test shows good antiradical activity. Antioxidant activity by the FRAP test, show that St total alkaloids of the roots have a value of 5.94 ± 0.14mmol EAA/g that is significantly close from Ascorbic acid (5.86 ± 0.51 mmol EAA/g). The different investigations show that Strychnos icaja has a good antioxidant activity and remarkable anti-inflammatory properties.

KEYWORDS:
Alkaloids; anti-inflammatory; antioxidant; polyphenols; Strychnos icaja.

ARTICLE HISTORY:
Received Oct 15, 2021
Accepted Nov 22,2021
Published Dec 17, 2021

DOI:
10.5455/ jcmr.2021.12.04.35

VOLUME: 12
ISSUE: 4
ISSN: 2146-8397

INTRODUCTION

Nature is full of many medicinal plants. Among these are poisonous plants that can be of great danger to humans or animals. Despite this toxicity, some plants may have therapeutic indications in the pharmacopoeia. We can cite as an example the ricin, of the Euphorbiaceous (Ricinus communis) which is a toxic plant, but which proves to be a powerful purgative and very irritating. Colchicum (Colchicum) is a genus of perennial herbaceous plant family Liliaceae that has therapeutic properties in the treatment of acute gout attack and as a muscle relaxant [1]. It is for this reason that we study the pharmacological properties of Strychnos icaja Baillon, a plant formerly used as a test poison, but which has therapeutic activity. The Strychnos icaja Baillon (Loganiaceae) is a vine that can reach a height of 20 to 40 m and a length of 20 to 100 m with a stem of 4 to 15 cm diameter [2]. Reign Plantae; order Gentianales. The Strychnos icaja Baillon occurs in the forests of Central Africa (Congo, Cameroon, Rwanda, and Gabon). It is a plant of dense and secondary forests [2]. The Strychnos icaja Baillon' s known vernacular names: Ikaza (Mpongwè), Ikadja kwaï (Benga), Kasé (Bakèlè), Mbundu (Galoa, Nkomi, Oroungu, Ngowé, Eshira, Bavarama, Bavoungou, Bapunu, Balumbu, Bavili, Baduma, Banzabi, Loango, Masangu, Mindumu), Mbondo (Ivea, Bavové, Bakota), Molela (Apindji), Mvya (mitsogo), Bilon (Fang) [3]. Root barks are used both as poisonous arrows and malaria treatment [4] by some pygmy tribes of Cameroon. In Gabon, root barks treat haemorrhoids in sitz baths. Dry root bark powder, mixed with palm oil, is applied topically to affected areas in dry or oozing

skin dermatoses [5]. It could be aphrodisiac at low doses and also used as an emetic, to" wash the belly" and against ear infections. In addition to its therapeutic use, the root is frequently used by populations for its pharmacological properties. The Mbundu is considered as the original judge, hence its usage as a test poison. In the Democratic Republic of Congo, macerated root rasp is used for measles treatment. Others usages are decoction of liana to cure beriberi, leaves' calcination for back pain and diabetes and leaves and roots decoction for epilepsy [6]. The toxicity of Strychnos icaja was attributed to the strychnine and hydroxystrychnine mono-indole alkaloids isolated from the roots by [7, 8, 9]. Strychnos icaja has been the subject of several studies, particularly on alkaloids from the roots of Strychnos icaja from Congo [10, 11]. These compounds, 18-hydroxy isosungucine mainly, are moderately active against Plasmodium falciparum. Another compound, Strychnogucin B, is cytotoxic against the human KB cell line of cancer and human fibroblasts WI38 [12]. Lusakibanza et al. [13], have also shown that the methanolic and dichloromethane extracts of the root bark had an excellent selectivity index against falciparum strains. In addition to this interesting antimalarial activity, it showed moderate in vitro antitrypanosomal activity [14]. This work was motivated by the scientific absence of work on polyphenols and the anti-inflammatory properties of Strychnos icaja. The present study aimed to establish the phenolic and alkaloids content and evaluate the in vitro anti-inflammatory and antioxidants activities of different extracts of the root of Strychnos icaja.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used were: Acetone (PubChem CID: 180), Butanol (PubChem CID: 263), Dichloromethane (PubChem CID: 6344), Ethyl acetate (PubChem CID: 8857), petrol benzene, methanol (Sigma Aldrich, Germany), DDPH (2, 2-diphenyl-1-picrylhydrazyl hydrate), ABTS (acid 2, 2-azimo-bis 3-éthylbenzothiazoline-6-sulfonic). Sulfuric acid (PubChem CID: 1118), ammonia (PubChem CID: 222), Dragendorff reagent (PubChem SID: 381001373), Folin–Ciocalteu reagents, potassium hexacyanoferrate [K3 Fe (CN) 6]. Quercetin hydrate (PubChem CID: 5280343), Allopurinol (PubChem CID: 135401907), Ascorbic Acid PubChem CID: 54670067)

Plant materiel

We used for testing, stems and roots of Strychnos icaja. Plants were harvested in the forest of the Cap Mondah Esterias. The identification of the plant material done in the field and at the National Herbarium of Gabon. Drying takes place in the shade at room temperature for 2-3 weeks. A voucher specimen was deposed in this department (Maesen et al., 5907; A.J.M. Leuwenberg.11470; J.M. and B. Reitsma.2137; R. Sita.5172).

Preparation of the plant extract

The spraying was done after drying the harvested plants. We used a flail chopper (Model SK 100) for the coarse powder. The study covered stems and roots of Strychnos icaja, and we crushed stems and roots separately. The root's aqueous extract is obtained by macerating 100 ml of it in 500 ml of water during 24 hours. After filtration, the marc is flipped twice. Filtrates were collected and frozen (Laborota 4002-Control Heidolph, Germany), then lyophilised.

Alkaloids extraction

The drug used is the stem and root powder for all tests. Introduce 100 g of dry, coarsely pulverised drug into a brewer. Add dilute sulfuric acid (1: 20 concentrated H2SO4 with distilled water) in a ratio of 5: 1 to 10: 1 (volume of acid: the weight of the plant) and then stop. Stir and let macerate for 24 hours at laboratory temperature. Filter on paper and wash with water to obtain about a filtrate. Then alkalinise the filtrate with ammonia to pH 8-9 and put in a separatory funnel. Add chloroform and stir without emulsification. After decantation, remove the organic phase. Repeat this operation until the alkaline solution no longer precipitates with the Dragendorff reagent. Combine the organic phases and dry over anhydrous sodium sulphate, filter and transfer to a calibrated evaporation flask to dry rotavapor under reduced pressure.

Polyphenols extraction

Strychnos icaja roots were cut into small piece and dried at room temperature for one month. Thereafter the barks were crushed into powered using a crusher (Retsch SK 100 Confort Geissen Germany). 100 g of the powered is defatted with petrol benzene, filtered and dried. The powder is taken with distilled water and evaporated, in a rotary evaporator (Buchi), to obtain the aqueous fraction. The same thing is doing with the methanol to get the methanolic fraction. In a second time, 50 g of powder macerated for 24 hours with acetone/water (80:20). After filtration, acetone is removed in a rotavapor at 40°C. The aqueous extract is taken with butanol and shook in a funnel. Then the butanol fraction is evaporated in a rotavapor at 40°C to obtain the butanol fraction. After that, the residue is shaken again with dichloromethane and evaporated to obtain the dichloromethane fraction. Finally, the last residue is shaken with ethyl acetate and evaporated to obtain the ethyl acetate fraction.

Determination of polyphenolic compounds
Determination of total polyphenols

The total phenolic content was determined using Folin-Ciocalteu reagents by the method of Slinkard and Singleton [15], with analytical grade gallic acid as the standard. 1 ml of extract or standard solution (0–500 mg/l) was added to deionised water (10 ml) and Folin–Ciocalteu phenol reagents (1.0 ml). After 5 minutes, 20% sodium carbonate (2.0 ml) wad poured to the mixture. After being kept in total darkness for one hour, the absorbance was measured at 760 nm using a spectrophotometer (CECIL 2041 Series UV / VIS, England). Amounts of total phenolic were calculated using a gallic acid calibration curve. The results are expressed in mg of gallic

equivalent per 1g of dry extract (mg Eq AG / g).

Determination of total flavonoids

The estimation of total flavonoid content is carried out by the method of Loots et al. [16]. 0.5 ml of the extract are mixed with 2 ml of distilled water and 0.15 ml of 15% $NaNO_2$ solution (sodium nitrite). After 6 minutes, 0.15 mL of 10% $AlCl_3$ is added and left for 6 minutes, then 2 mL of 4% NaOH (sodium hydroxide) is added. The volume is adjusted to 5 mL with distilled water. The absorbance is measured after 15 minutes at 510 nm. The concentration of the flavonoids is calculated by referring to a calibration curve obtained using quercetin as a standard. The results are expressed in milligrams equivalent of quercetin for 1 g of dry extract (mg EQ / g).

Determination of total tannins

This assay was performed according to the method proposed by the FAO [17]. Briefly, 1 ml of extract to be assayed is mixed with 5 ml of water vortex which is added 1 ml of ferric ammonium citrate (28% iron, 3.5 g / l) (about 24 hours) and 1 ml ammonia (8 g / l). The absorbance of the solution is measured at 525 nm after 10 min against a blank (1 ml of extract dose + 6 ml of water + 1 ml of ammonia) for three readings. The tannic acid was used as a standard to plot the calibration curve. The results are expressed in milligrams of tannic acid equivalent per 1 g of dry extract (mg EAT / g).

Anti-inflammatory activity by inhibition of lipoxygenase

The method consists of inhibiting the lipoxygenase cycle (LOX), thus preventing the production of leukotriene and lipoxin. Leukotrienes, generated by lipoxygenase cycle are inflammatory and known to be potent mediators, plays an important role in allergic reactions. They may also be involved in ischemia and atherosclerosis. Cerebral vascular accident, traumatic brain injury and Alzheimer's, disease have also been linked to the activity of lipoxygenase cycle, especially the 5-LOX and leukotrienes. It has been shown that lipoxygenase produced in the body plays an important role in respiratory disorders such as bronchial asthma, inflammation [18, 19]. Inhibit the lipoxygenase cycle is a way of preventing treatment disorders of those diseases. The inhibitory activity of lipoxygenase extracts was determined by the spectrophotometric method [20] with some modifications. The percentage of inhibition was calculated using the formula:

$$I\ \% = (E - S)/E \times 100$$

E is the enzyme activity without inhibitor and S, enzyme activity in the presence of the inhibitor.

Ant-inflammatory activity by inhibiting xanthine oxidase

Xanthine oxidase is a metabolic pathway of the formation of uric acid. The xanthine oxidase inhibitor is used in the treatment of gout caused by an accumulation of uric acid in humans. Xanthine oxidase or xanthine dehydrogenase is an enzyme catalysing the oxidation of hypoxanthine to xanthine and further catalyses the oxidation of xanthine to uric acid. The method Ferraz et al. [21] was used with some modifications.

Antioxidant activities

Reducing powered with FRAP method

The FRAP method (Ferric Reducing Antioxidant Power) is based on the ability of extracts to reduce ferric ion (Fe^{3+}) by ferrous ion (Fe^{2+}). Total antioxidant capacity of each plant extract was determined by the method Benzie and Strain [22]. And 1 ml of an aqueous solution of each extract (10 mg/ml diluted 100th for 0.1 mg/ml), ascorbic acid, was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After 30 min of incubation at 50 ° C, 2.5 ml of trichloroacetic acid (10%) was added. The mixture was then centrifuged at 3000 rpm/min for 10 min. 2.5 ml of the supernatant were then mixed with the same volume of water, and 0.5 ml of a freshly prepared aqueous solution of $FeCl_3$ (0.1%) was added. The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-100 mg / l). The reducing power is expressed in ascorbic acid equivalent (AAE) (mmol ascorbic acid / g of dry extract).

Antiradical activity by the method of inhibition of DPPH radical

The antiradical activity of plant extracts reflects their ability to scavenge free radicals from the body. The radical scavenging activity was evaluated on the different fractions of Strychnos icaja. The extracts were dissolved in methanol to obtain concentrations of mothers of 10 mg/ml. This concentration is diluted to 100 for the second test. The method spectrophotometric 2.2-diphenyl-1-picrylhydrazyl (DPPH) as described by Popovici et al. [23] is used with some modifications. Introduce 1.5 ml of a methanol solution of DPPH at 20mg / l in test tubes containing 0.75 ml of extracts prior to the test. A control containing no plant extract is also prepared. The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-200 mg / l). Each test was performed in triplicate. Antiradical power was expressed as ascorbic acid equivalent (AAE) (mmol ascorbic acid / g of dry extract). The concentration of reducing compounds (antioxidants) in the extract is expressed in mmol ascorbic acid equivalent (AAE) / g of dry extract.

Antiradical activity by the method of inhibition of the radical cation ABTS

The method described by Pellegrini et al. [24] is used. It is based on the discolouration of a stable radical cation, ABTS + (2.2 '-azinobis- [3-acid-6-sulfonic ethylenzothiazoline]) to ABTS in the presence of antioxidant compounds at 734 nm. The radical cation ABTS + was generated by reacting an aqueous solution of ABTS (7 mM) with 2.5 mM potassium persulfate (final concentration), the mixture is kept in the dark at room temperature for 12 hours before use. The mixture was diluted

with ethanol to give an absorbance of 0.70 ± 0.02 to 734 nm using the spectrophotometer. For each extract, a methanol solution (10 mg/ml) is diluted to 100th in ethanol µl of sample and 10 (solution), the reference substance (ascorbic acid) were mixed with 990 µl of a fresh solution of ABTS +. The set is stored away from light for 15 minutes, and absorbances were read at 734 nm in a spectrophotometer against a standard curve of ascorbic acid precisely 6 min after initial mixing. The concentration of compounds having a reducing effect on the radical cation ABTS + is expressed in mmol ascorbic acid equivalent (AAE) / g of dry extract. The concentration of reducing compounds (antioxidants) in the extract is expressed in mmol ascorbic acid equivalent (AAE) / g of dry extract.

Statistical analysis

All data represent the average of three trials. For the comparison of the results, analysis of the variance, ANOVA and Dunnett test (GraphPad Instat) are used, and the degree of data significance is taken at the probability $P \leq 0.05$.

RESULT

Phytochemical studies

Dosage of alkaloids

Extraction of alkaloids allowed us to obtain the following levels in roots (0.660 %), stems (1.5792 %) and leaves (2.5 %).

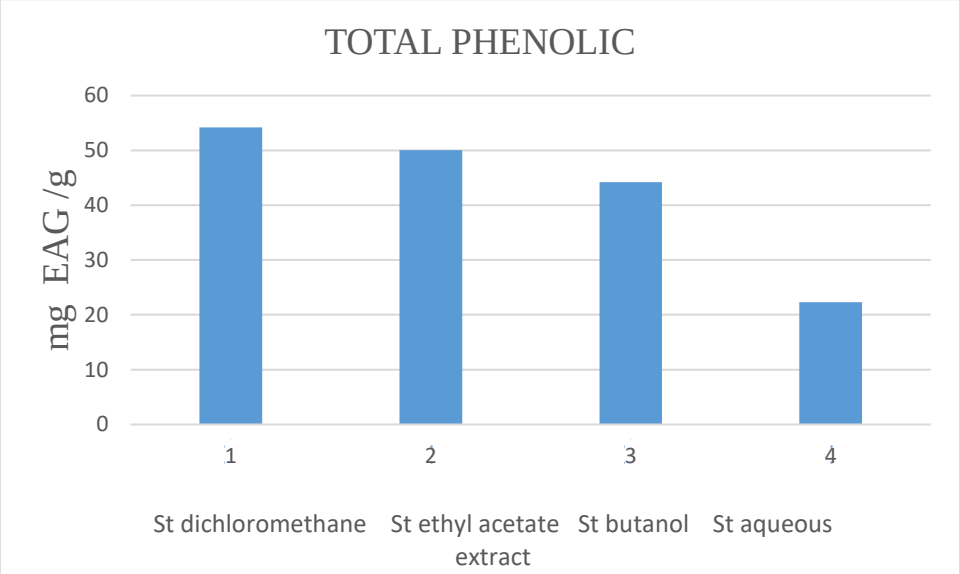
Determination of extracts polyphenols compounds

Dosage of phenolic compounds

The dosage of phenolic compounds has shown the rates of 54.21 ± 4.87 mg EAG / g for St dichloromethane; 50.03 ± 2.00 mg EAG / g for St ethyl acetate; 44.20 ± 0.69 mg EAG / g for St butanol and 22.28 ± 0.50 mg EAG / g for St aqueous root extract. (Table I, Graph 1).

Table 1: Demonstration of total phenolics, total flavonoids and total tannins in various extracts of Strychnos icaja root

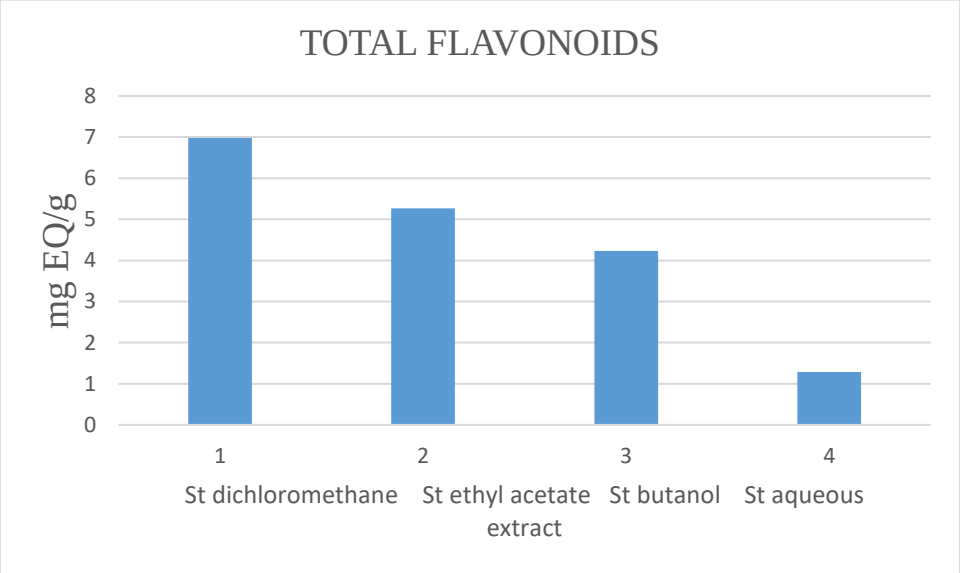
Types of extracts	Total Phenolic (mg EAG/g)	Total flavonoids (mg EQ/g)	Total tannins (mg EAT/g)
St dichloromethane	54.21±4.87	6.98±1.27	46.42±1.03
St ethyl acetate	50.03±2.00	5.27±1.20	41.88±0.67
St butanol	44.20±0.69	4.23±0.08	38.02±0.66
St aqueous extract	22.28±0.50	1.29±0.45	17.30±0.69



Graph 1: Determination of total phenolic in various extracts of Strychnos icaja root

Dosage of flavonoids compounds

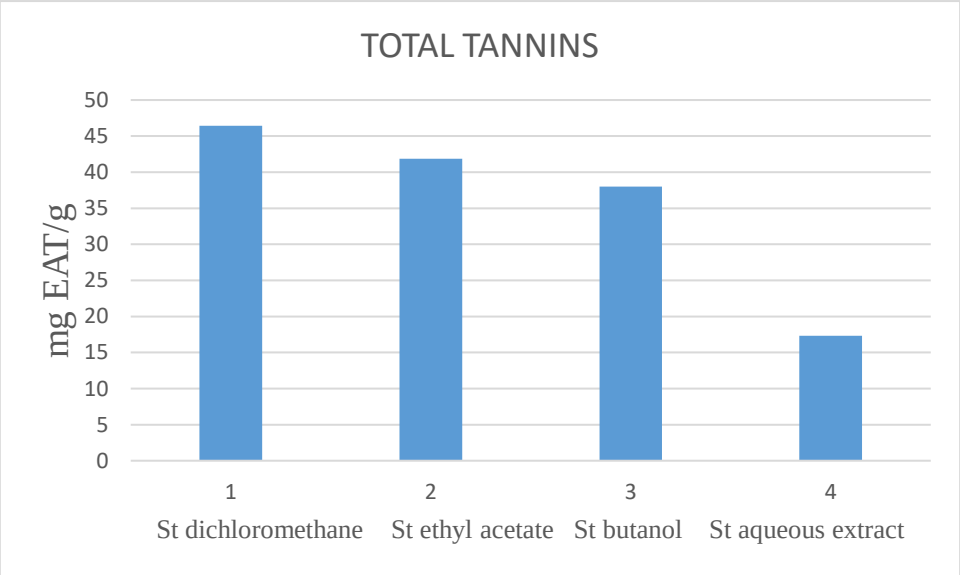
The dosage of flavonoids compounds has shown the rates of 6.98 ± 1.27 mg EQ/g for St dichloromethane; 5.27 ± 1.20 mg EQ/g for St ethyl acetate; 4.23 ± 0.08 mg EQ/g for St butanol and 1.29 ± 0.45 mg EQ/g for St aqueous root extract (Table I, Graph 2).



Graph 2: Determination of total flavonoids in various extracts of Strychnos icaja root

Dosage of total tannins

The dosage of tannins compounds has shown the rates of 46.42±1.03 mg EAT/g for St dichloromethane; 41.88±0.67 mg EAT/g for St ethyl acetate; 38.02±0.66 mg EAT/g for St butanol; 17.30±0.69 mg EAT/g for St aqueous root extract (Table I, Graph 3).



Graph 3: Determination of total tannins in various extracts of Strychnos icaja root

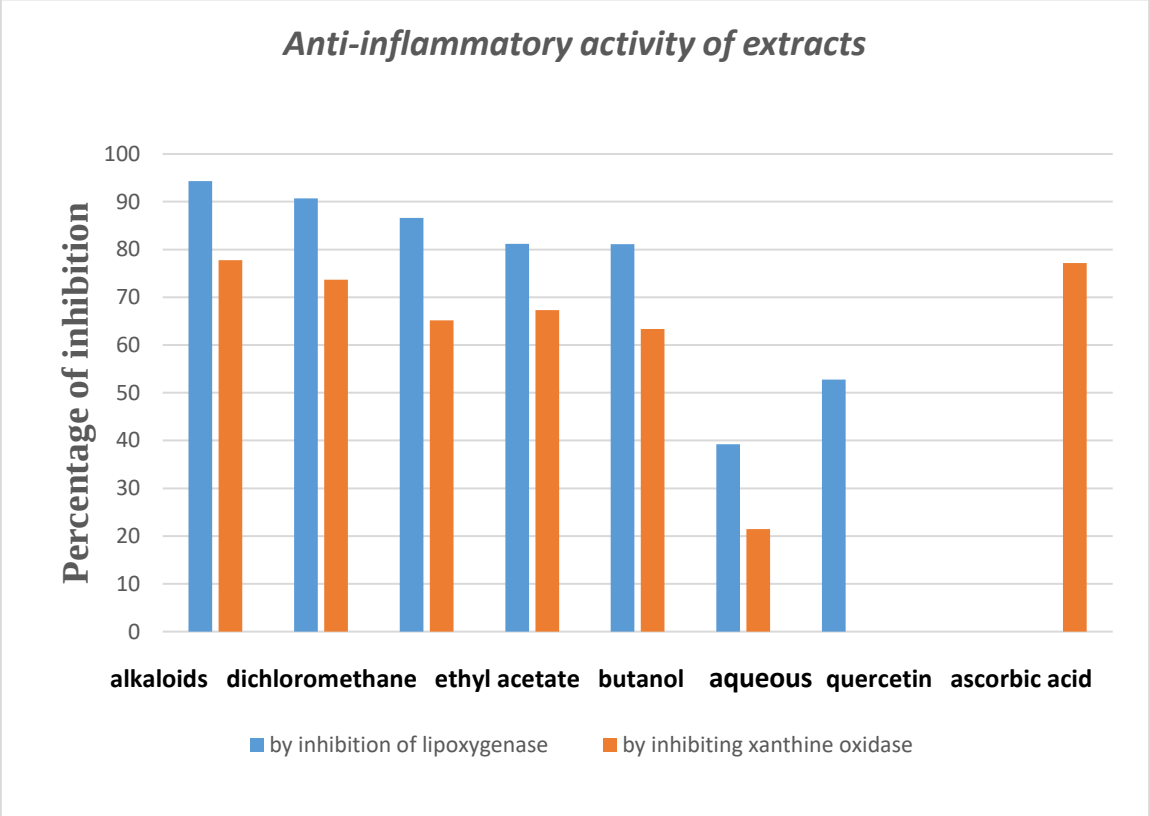
Anti-inflammatory activity of extracts

We used two reference substances (quercetin and allopurinol) by the reaction to lipoxygenase and xanthine oxidase. Table II, Graph 4, shows the anti-inflammatory activity of our extracts. For the lipoxygenase method, the statistical analysis of the variances shows that the reference value of quercetin (52.74 ± 1.72 %) is clearly different from the values obtained with the different extracts of Strychnos icaja according to the comparison test Dunnett's multiple ($p < 0.01$). For St total alkaloids roots we are obtained 94.33 ± 0.16 %; for St dichloromethane roots 86.61±2.83 %; for St ethyl acetate roots 81.18±0.53 %; for St butanol roots 81.11±1.03 %: the extract that has the smallest percentage inhibition is St aqueous extract roots (39.25 ± 3.22 %). For the xanthine oxidase method, the ANOVA test indicates that the reference value of allopurinol (77.13 ± 0.41%) is not significantly different from the values obtained with St total alkaloids roots according to Dunnett's multiple comparison test ($P > 0.05$). We are obtained for St total alkaloids roots, 77.74 ± 0.14 %; for St dichloromethane roots 65.19±5.25 %; for St ethyl acetate roots 67.33±7.57 %: for St butanol roots 63.33±.58 %; the extract that has the smallest percentage inhibition is St aqueous extract roots (21.49 ± 1.05 %), $p < 0.01$.

Table 2 : Evaluation of the anti-inflammatory activity of Strychnos icaja extracts in comparison with reference substances: quercetin and allopurinol by the action of lipoxygenase and xanthine oxidase

Types of extracts	LYPOXYGENASE (% inhibition)	XANTHINE OXIDASE (% inhibition)
St total alkaloids roots	94.33±0.16**	77.74±0.14
St dichloromethane roots	86.61±2.83**	65.19±5.25
St ethyl acetate roots	81.18±0.53**	67.33±7.57
St butanol roots	81.11±1.03**	63.33±.58
St extracted aqueous roots	39.25±3.22**	21.49±1.05**
Quercetin (50 µg/ml)	52.74±1.72	Nd
Allopurinol (50 µg/ml)	Nd	77.13±0.41

NB: ** p <0.01: the difference is very significant
* p <0.05: the difference is significant
Nd: not determined



Graph 4: Anti-inflammatory activity of extract by inhibition of lipoxygenase and xanthine oxidase

Antioxidant activities extracts

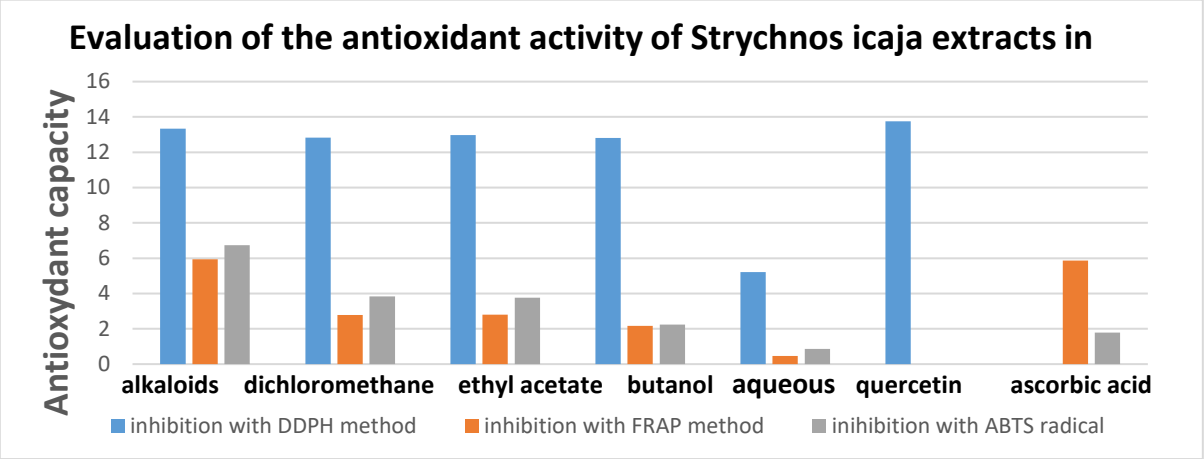
Antioxidant activities by inhibition of DPPH

Antiradical activity by the method of inhibition of DPPH radical has a content of 13.33 ± 0.16 mmol AAE/g for St total alkaloids roots; 12.82 ± 0.10 mmol AAE/g for St dichloromethane; 12.97 ± 1.05 mmol AAE/g for St ethyl acetate; 12.80 ± 0.04 mmol AAE/g for St butanol; 5.21 ± 0.01 mmol AAE/g for St aqueous root extract and 13.76 ± 0.26 mmol AAE/g for quercetin (Table III, Graph 5).

Table 3: Evaluation of the antioxidant activity of Strychnos icaja extracts in comparison with quercetin and ascorbic acid by different methods

Extract type	DPPH (mmol AAE/g)	FRAP (mmol AAE/g)	ABTS (mmol AAE/g)
St alkaloids total roots	13.33±0.16	5.94±0.14	6.74±0.14**
St dichloromethane roots	12.82±0.10	2.78±0.10**	3.83±0.15**
St ethyl acetate roots	12.97±1.05	2.81±0.01**	3.77±0.20**
St butanol roots	12.80±0.04	2.16±0.06**	2.24±0.57
St extracted aqueous roots	5.21±0.01**	0.46±0.27**	0.86±0.25
Quercetin	13.76±0.26	Nd	Nd
Ascorbic acid	Nd	5.86±0.51	1.78±0.58

NB: ** p <0.01: the difference is very significant
* p <0.05: the difference is significant
Nd: not determined



Graph 5: Antioxidant activity by the method of inhibition of DPPH, with FRAP method, by inhibition of cation ABTS

Reducing powered with FRAP method

Reducing powered with FRAP method has a content of 5.94 ± 0.14 mmol AAE/g for St total alkaloids roots; 2.78 ± 0.10 mmol AAE/g for St dichloromethane; 2.81 ± 0.01 mmol AAE/g for St ethyl acetate; 2.16 ± 0.06 mmol AAE/g for St butanol; 0.46 ± 0.27 mmol AAE/g for St aqueous root extract and 5.86 ± 0.51 mmol AAE/g for ascorbic acid (Table III, Graph 5).

Antiradical activity by inhibition of ABTS +

Antiradical activity by the method of inhibition of the radical cation ABTS + has a content of 6.74 ± 0.14 for St total alkaloids roots, 3.83 ± 0.15 for St dichloromethane, 3.77 ± 0.20 for St ethyl acetate, 2.24 ± 0.57 for St butanol, 0.86 ± 0.25 for St aqueous root extract and 1.78 ± 0.58 for ascorbic acid (Table III, Graph 5).

DISCUSSION

Strychnos icaja has been the subject of several studies, particularly on alkaloids from the roots of Congo [25, 26] from which they have isolated active compounds against Plasmodium falciparum. Lusakibanza M. et al. [27], have also shown that the methanolic and dichloromethane extracts of the root bark had an excellent selectivity index against falciparum strains. In addition to this interesting antimalarial activity, it showed moderate in vitro antitrypanosomal activity [28]. This confirms their use in the treatment of malaria by pygmies. The dosage of alkaloids is different according to the part of the plant harvested. Their extraction allowed us to obtain the following rates in the roots (0.660%), the stems (1.5792%) and the leaves (2.5%). Long before Denoel [29], in 1950 found up to 10 to 16% alkaloids in barks of stems and roots and 1 to 2% in the leaves. The plant also contains flavonoids and tannins. Flavonoids are part of the polyphenol family. They are present in plants and are mainly used to give colour to plants. Flavonoids are recognised like antitumor, antiviral, anti-allergy, anti-inflammatory [30] and vascular protective more antiseptic or antibacterial. Many studies indeed suggest that flavonoids have anti-inflammatory [31] properties and can modulate the immune system. The determination of phenolic compounds tells us that the different extracts are much richer in tannins than flavonoids. Depending on their concentration, the tannins have the capacity to tighten the tissues and modify the membrane permeability by complexing with their glycoprotein chains [32], hence their internal use (antidiarrheal, antitumor, anti-inflammatory action, antimicrobial, antifungal and antiviral activity) and external (haemostatic effect, healing) [33]. This confirms their use by populations in the treatment of haemorrhoids [2] and in the treatment of measles. Anti-inflammatory activity with quercetin (52.74 ± 1.72) reference substance shows a high percentage inhibition of lipoxygenase for St alkaloids root extract (94.33 ± 0.16) and with allopurinol (77.13 ± 0.41) substance reference, St alkaloids root extract have significantly the same percentage of inhibition (77.74 ± 0.14). These results confirm that Strychnos icaja possess a high anti-inflammatory effect. Values show that higher the tannin content, more anti-inflammatory activity is high. This explains why St aqueous extract has the smallest percentage. This study shows why this specie is used by the locals to treat diseases like back pain. Beyond the anti-inflammatory properties of our extracts, we evaluated their antioxidant capacity because antioxidants are documented in several publications to mitigate the inflammatory processes and this plants activity has been ascribed to the phenolic compounds present in it, particularly flavonoids and tannins [34, 35, 36, 37]. Antiradical activity by the method of inhibition of DPPH radical shows that St alkaloids root and St alkaloids stem have the best inhibition of radical DPPH followed with St dichloromethane, St ethyl acetate, St butanol and much less for St root aqueous extract. Those values except St root aqueous extract are significantly close to that quercetin (13.76 ± 0.26 mmol AAE/g). Reducing powered with FRAP method show the same result, with high value to St alkaloids root and low value to St aqueous extract compared to ascorbic acid (5.86 ± 0.51 mmol AAE/g). The FRAP test makes it possible to evaluate the antioxidant power of foods by determining this capacity for reducing ferric ions to ferrous ions. The antioxidant content is determined by the solutions containing the known concentrations of ferrous ions. FRAP is expressed in mmol of antioxidants per 100 g of food.

Here is how to interpret the FRAP values of foods: 0 <FRAP index <1.5: low antioxidant capacity; 1.5 <FRAP index <3: average antioxidant capacity; 3 <FRAP index <10: high antioxidant capacity; FRAP index> 10: very high antioxidant capacity [38]. So, St alkaloids total roots have a high antioxidant capacity and St aqueous extract a low antioxidant capacity extract. Antiradical activity by the method of inhibition of the radical cation ABTS show high value compared to ascorbic acid reference substance (1.78 ± 0.58 mmol AAE/g). Free radicals are harmful substances generated by the body that damage cells, causing premature ageing and the development of certain diseases. Thus, the role of antioxidants is to combat the oxidation caused by these free radicals. However, they are more or less held in check by the body's natural antioxidants. Some factors can, however, break this balance. If free radicals come to exceed the body's ability to neutralise them, they can contribute to the onset of many diseases, including cardiovascular disease, certain types of cancer and other diseases associated with ageing [39]. Lansiaux et al. [40] have isolated from Strychnos icaja root, and a compound named Strychnogucin B, who's cytotoxic against the human KB cell line of cancer and against human fibroblasts WI38. Many chemicals in foods are called antioxidants because they have the property of preventing harmful chain reactions caused by free radicals. They are bulletproof for the body. The main natural antioxidants are bioflavonoids, carotenoids, vitamins C and E, and selenium [41]. Compared to quercetin or vitamin C, our extracts have excellent antioxidant activity. In view of the results presented in Table II, we find that the plants richest in phenolic compounds have the highest antioxidant activity. These results are consistent with what is reported in the literature by several authors that the potential for antioxidant activity of an extract depends on its content of phenolic compounds [42, 43, 44, 45, 46]. Strychnos icaja has a very good anti-inflammatory activity, which justifies its use despite its high toxicity.

CONCLUSION

The purpose of this work was to evaluate the antioxidant and anti-inflammatory properties of Strychnos icaja Baillon (Loganiaceae), a plant formerly used as a test poison. These roots are also used to treat various conditions, such as back pain, haemorrhoids, and measles. The dosage of this plant confirms that it is rich in tannins that are compounds capable of trapping free radicals and thus reduce oxidative stress. The evaluation of the anti-inflammatory activity revealed that the Strychnos icaja Baillon possesses an anti-inflammatory power significantly close to that of allopurinol by the method of inhibition of xanthine oxidase and superior to quercetin by the method of inhibition to lipoxygenase. This work confirms the traditional usage done by the autochthones.

COMPETING INTERESTS

The authors declare that they have no competing interests

ACKNOWLEDGEMENTS

The authors wish to thank Mrs Aworet Maky Emiliana Tiffany for her help in assistance by reviewing the manuscript. This work was supported by IPHAMETRA/CENAREST Gabon. All procedures in this study are in accordance with the Gabonese laws and ethical rules.

REFERENCES

1. Fournier PV. Dictionnaire des plantes médicinales et vénéneuses de France. Presses de la Cité, 2010 ; 1048 pages.
2. Aubreville A, Jean F. Flore du Gabon-19-loganiacées. Muséum National d'Histoire Naturelle, Paris (France). 1972 ; 153 p.
3. Walker R, Sillans. Les Plantes Utiles du Gabon, 1961. Editions Paul Lechevalier, Paris.614 p.
4. Neuwinger HD. African Ethnobotany, Poisons and Drugs. Chapman & Hall, 1996; London p569-576.
5. Adjanohoun EJ, Aké Assi L, Chibon P, De Vecchy H, et al. Contribution aux études ethnobotaniques et floristiques au Gabon. Col Médecine traditionnelle et pharmacopée, 1984, ACCT.294p.
6. Konda Ku Mbuta C, Kabakura M , Mbembe B, Itufa Y, Mahuku K., Mafutam M, et al. Medicinal plants of traditions Province of Ecuador, 2012, p326.
7. Sandberg F, Roos K, Ryrberg KJ, Kristiansson K. A new alkaloïd, 4- hydroxystrychnine, from African specie Strychnos icaja Baillon. Tetrahedron Lett. 1968; 59, 6717-8.
8. Sandberg F, Roos K, Ryrberg KJ, Kristianson K. The pharmacologically active alkaloids of Strychnos icaja Baillon strychnine and a new alkaloïd, 4-hydroxystrychnine. Acta Pharm Suec. 1969; 6,103-8.
9. Sandberg F, Kristianson K. A comparative study of the convulsant effects of Strychnos alkaloids. Acta Pharm.Suec.1970; 7, 329-336.
10. Frédérich, De Pauw MC, Llabrès G, Tits M, Hayette MP, Brandt V, Penelle J, De Mol P, Angenot L. New antimalarial and cytotoxic sungucine derivatives from Strychnos icaja roots.Planta Med. 2000 ; Apr; 66(3):262-9.
11. Frédérich M, De Pauw M, Prosperi C, Tits M, Brandt V, Penelle J, Hayette M, DeMol P, Angenot L. Strychnogucines A and B, two new antiplasmodial bisindole alkaloids from Strychnos icaja. J Nat Prod. 2001; Jan; 64(1):12-6.
12. Lansiaux A, Bailly C, Houssier C, Colson De Pauw-Gillet MC, Frederich M, Tits, M, Angenot L. Apoptosis of HL-60 leukemia cells induced by the bisindole alkaloids sungucine and isosungucine from Strychnos icaja. Planta med. 2002 ; 68, 591-5.
13. Lusakibanza M, Mesia G, Tona G, Karemere S, Lukuka A, Tits M, Angenot L, Frédérich M. In vitro and in vivo antimalarial and cytotoxic activity of five plants used in Congolese traditional medicine. J Ethnopharmacol. 2010; Jun 16; 129(3):398-402.
14. Beaufay C, Ledoux A, Jansen O, Bordignon A, Zhao S, Teijaro CN, Andrade RB, Quetin-Leclercq J, Frédérich M. In vivo Antimalarial and Antitrypanosomal Activity of Strychnogucine B, a Bisindole Alkaloid from Strychnos icaja. Planta Med. 2018; Aug; 84(12-13):881-885.
15. Slinkard K, Singleton VL. Total phenol analyses automation and

comparison with manual methods. Am. J. Enol. Vitic. 1997 ; 28, 49–55.

16. Loots D, Francois H, Westhuizen V, Botes L. Aloe ferox leaf gel phytochemical content, antioxidant capacity, and possible health benefits. J. Agriculture Food Chemestry. 2007; 55: 6891 -6896.
17. FAO/IAEA Working Document IAEA, VIENNA. Quantification of Tannins in Tree Foliage. 2000; pp 3-4.
18. Steinhilber D. 5-Lipoxygenase: a target for anti- inflammatory. Drugs revisited. Curr Med Chem. 1999; 6:71-85.
19. Vidal C, Gomez HA, Sanchez GE, Gonzalez A, Ortega L, Gomez GJA, et al. Licofelone, a balanced inhibitor of cyclooxygenase and 5-lipoxygenase, reduces inflammation in a rabbit model of atherosclerosis. J Pharmacol Exp Ther. 2007; 320:108-16.
20. Malterud KE, Rydland KM. Inhibitors of 15- lipoxygenase from orange peel. J Agric Food Chem. 2000; 48:5576-80.
21. Ferraz FZS, Vitolo IF, Fietto LG, Lombardi JA, Saude GDA. Xanthine oxidase inhibitory activity of Lychnophora species from Brazil ("Arnica"). J Ethnopharmacol. 2006; 107:79-2.
22. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power": The FRAP assay. Analytical Biochemistry. 1996 ; 239, 70–76.
23. Popovici C, Saykova I, Tylkowski B. Evaluation de l'activité anti oxydante des composés phénoliques par la réactivité avec le radical libre DPPH. Revue de Génie Industriel. 2009 ; 4, 25-39.
24. Pellegrini Re R, Proteggente NA, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. And Med. 1999; 26, 1231-1237.
25. Frédérich M, De Pauw MC, Llabrès G, Tits M, Hayette MP, Brandt V, Penelle J, De Mol P, Angenot L. New antimalarial and cytotoxic sungucine derivatives from Strychnos icaja roots. Planta Med. 2000; Apr; 66(3):262-9.
26. Frederich M, De Pauw M, Prosperi C, Tits M, Brandt V, Penelle J, Hayette M, De Mol P, Angenot L. Strychnogucines A and B, two new antiplasmodial bisindole alkaloids from Strychnos icaja. J Nat Prod. 2001; Jan; 64(1):12-6.
27. Lusakibanza M, Mesia G, Tona G, Karemere S, Lukuka A, Tits M, Angenot L, Frederich M. In vitro and in vivo antimalarial and cytotoxic activity of five plants used in Congolese traditional medicine. J Ethnopharmacol. 2010; Jun 16; 129 (3):398-402.
28. Beaufay C, Ledoux A, Jansen O, Bordignon A, Zhao S, Teijaro CN, Andrade RB, Quetin-Leclercq J, Frederich M. In vivo Antimalarial and Antitrypanosomal Activity of Strychnogucine B, a Bisindole Alkaloid from Strychnos icaja. Planta Med. 2018 ; Aug;84(12-13):881-885.
29. Denoël, 1950. Le Strychnos icaja Baillon du Congo Belge. Journal de Pharmacie de Belgique, Bruxelles . 1950 ; Vol.5 No.3/4 pp.59-77.
30. Hadi M. La quercétine et ses dérivés: molécules à caractère peroxydant ou thérapeutiques. Thèse de doctorat. 2004 ; Université Louis Pasteur Strasbourg I. p 155.
31. Macheix JJ, Fleuriet A, Jay AC. Les composés phénoliques des végétaux. Presses polytechniques et universitaires romandes. Sciences des aliments. 2006 ; 26:189-90.
32. Paris M, Hurarielle M. Abrégé de matière médicale. Masson. 1981; 339p
33. Kerry B, Simon M. Principles and Practice of Phytotherapy-E-Book: Modern Herbal Medicine, Elsevier Health Sciences. 2013; p. 35-38.
34. Pereira NA, Pereira BMR, DO Nascimento MC, Parente JP, Mors WB. Pharmacological screening of plants recommended by folk medicine as snake venom antidotes; IV. Protection against Jararaca venom by isolated constituents. Planta Med. 1994; 60: 99-00.
35. Hutt MJ, Houghton PJ. A survey from the literature of plants used to treat scorpion stings. J Ethnopharmacol. 1998; 60: 97-10.
36. Sakai A, Hirano T, Okazai R, Okimoto N, Tanaka K, Nakamura T. Large-dose ascorbic acid administration suppresses the development of arthritis in adjuvant –infected rats. Arch Orthop. Trauma Surg. 1999; 119: 121-126.
37. Ozgov AS, Hermanek J, Gut I. Different antioxidant effects of polyphenols on lipid peroxidation and hydroxyl radicals in the NADPH-, Fe-ascorbate- Fe-microsomal systems. Biochem. Pharmacol. 2003; 66: 1127-1137.
38. Pouyat-Leclère J. Guide des aliments antioxydants. Thierry Souccar Editions. 2013.
39. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem. 2004; Jun 16; 52(12):4026-37.
40. Lansiaux A, Bailly C, Houssier C, Colson De Pauw-Gillet MC, Frederich M, Tits M, Angenot L, 2002. Apoptosis of HL-60 leukemia cells induced by the bisindole alkaloids sungucine and isosungucine from Strychnos icaja. Planta med. 2002; 68, 591-5.
41. Davalos A, Gomez-Cordoves C, Bartolome B. Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. J Agric Food Chem. 2004; Jan 14;52 (1):48-54.7
42. Amri O, Elguiche R, Tahrouch S, Zekhnini A, Hatimi A. Antifungal and antioxidant activities of some aromatic and medicinal plants from the southwest of Morocco. Journal of Chemical and Pharmaceutical Research. 2015; 7(7): 672-678.
43. Guettaf S, Abidli N, Kariche S, Bellebcir L, Bouriche H. Phytochemical screening and antioxidant activity of aqueous extract of Genista saharae (Coss. & Dur.). Der Pharmacia Lettre. 2016; 8 (1):50-60.
44. Kalia K, Sharma K, Singh HP, Singh B. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of Potentilla atrosanguinea Lodd and quantification of its phenolic constituents by RP-HPLC. J. Agric. Food Chem. 2008; 56: 10129–10134.
45. Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K. Screening for antioxidant activity in edible plant products: Comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay and Folin–Ciocalteu assay. J Agri Food Chem. 2004; 52: 2391–2396.
46. Stagos D, Portesis N, Spanou C, Mossialos D, Aligiannis N, Chaita E. Correlation of total polyphenolic content with antioxidant and antibacterial activity of 24 extracts from Greek domestic Lamiaceae species. Food and Chemical Toxicology. 2012 ; 50: 4115-412.