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# ANTITUSSIVE AND EXPECTORANT EFFECTS OF EXTRACTS, SOLUBLE FRACTIONS AND CRUDE POLYSACCAHRIDES, ACUTE AND SUBACUTE TOXICITY OF AQUEOUS EXTRACT FROM *SECURIDACA LONGEPEDUNCULATA* FRESEN (POLYGALACEAE) ROOTS IN ANIMAL MODEL

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## ABSTRACT

*Securidaca longepedunculata* roots Fresen (Polygalceaea) is used in Congolese traditional medicine as a remedy in aqueous decoction form to treat different coughs and bronchial asthma. In the present study, antitussive and expectorant activities of aqueous (decoction) and 80% methanol extracts, chloroform, etylacetate, *n*-butanol and residual aqueous soluble fractions from the partition of aqueous extract and crude polysaccharides were investigated by different classical models using different stimulus like amomonium hydroxide liquor, citric acid, sulphur dioxide and capsaicin-induced cough in animal model. The antitussive activity was evaluated by the appreciation of effects of tested samples and Codeine phosphate used as antitussive reference drugs on the number of cough frequencies and latent period of cough compared to negative control receiving 3% Tween 80 in their treatment while the expectorant activity was based on the capacity of tested samples to increase de production of tracheal phenol red. Results revealed that all tested samples from *Securidata longepedunculata* roots, at oral administered doses of 100 and 200 mg body weight, showed statistically and significant dose-dependent reduction of the number of cough frequencies and the latent period of cough caused by the exposure of treated rats to ammonia, citric acid in guinea pig, sulphur dioxide and capsaicin compared to negative control in all antitussive *in vivo* and exerted thus their antitussive activity. Similary, extracts and soluble fractions from *Securidaca. longepedunculata* increase significantly tracheal phenol red and showed their expectorant activity compared to negative control. 80% Methanol extract showed high activities compared to aqueous extract and ethylacetate soluble fraction exhibited high activities compared to other soluble fractions like chloroform, *n*-butanol and residual aqueous. Both activities of extracts and soluble fractions from were weak compared to Codeine phosphate used as reference antitussive product.

**KEYWORDS:** *Securidaca longepenculata*, Polygalaceae, roots, extracts, soluble fractions, antitussive and expectorant activities.

## 1. INRODUCTION

Medical sciences describe cough as a defensive reflex of the respiratory tract and is considered for human body to clear upper airways unless it becomes aggraved and interfere with the normal respiration (Poliverino et al., 2012; Billah et al., 2021).

Coughing is also seen as a symptom of respiratory diseases that prevents talking and causes chest and thorax pains (Irwin and Madison, 2000; Guo et al., 2016). It is a sudden and forceful expiration of air from the lungs caused by involuntary contractions of the muscles controlling the processes on breath (Hircnjal et al., 2013; Petros et al., 2020). It is a physiological reflex process to remove foreign materials and secretions from airways.

Cough is the most common symptom of various airway inflammatory diseases such as bronchitis, bronchial asthma, chronic obstructive pulmonary diseases (COPD) and lung cancers (Nasra and Belvisi, 2009; Wu et al., 2018; Petros, 2020). It could be also considered as a symptom of extra-pulmonary conditions like gastro-eosophageal reflex disease (GERD) or heart disorders

and could be due to side effects of certains used drugs or medications i.e angiotensin-converting enzyme inhibitors or without any associated causes, often refered to idiopathic coughs (Gupta et al., 2009; Reynolds et a., 2009; Petros et al., 2020).

Indeed, acute cough can be caused by viral upper respiratory tract infection accounts for enormous expenditure on prescription and non-prescription cough-relief products worldwide and the suppression of cough by antitussive dugs is need in many pathological conditions, particularly if no other causal treatment is possible such as the case of idiopathic chronic coughs (Kahas et al., 2018). Coughing is an essential and important defensive mechanism as well as a typical sign of diseases such as cancer obstructive pulmonary disease (COPD) and asthma. The cough reflex can be intentionally provoked and aids in clearing the airways of extra secretions and foreign objects (Pal et al., 2024).

A respiratory disease called cough raises the risk of recidivism. Despite the development and availability of antitussive drugs, the conditions continue to represent a significant risk to health. The most common and currently antitussive medications are known to have many side effects that limit the effectiveness of cough treatment, and include increased sputum viscosity, depression of the respiratory centre, decreased bronchial secretion, inhibition of ciliary activity, hypotension, decreased expectoration and constipation (Pal et al., 2024).

Dry and unproductive cough are often linked with eosinophilic bronchitis, airways irritations as results of air polluants, airways, allergies, gastro-enterophageal reflux diseases (GERD) (Gupta et el., 2009; Lai et al., 2013; Billah et al., 2021). Cough apart from being traditionally classified as either productive, i.e producing mucus usually with expectoration, and non-productive or dry cough, can also be mentioned on the duration into acute, subacute and chronic coughs (Petros et al., 2020).

Treating of cough mainly consist of the treating the underlying causes. Hydratation of respiratory tract by steam inhalation and demulcents is helphul in reducing majority of cough symptoms (Padma, (2013), Gupta et al., (2014); Petros, 2020) or is effective in reducing symptoms in the majority of case, but, for uncontrolled cough, opioidergic central cough suppressants are used. However, their greatest disadvantage is a high rate of unwanted effects such as depression of respiratory centre, decreased secretion in the bronchioles and inhibition of ciliary activity as already mentioned above (Paneliya et al;, 2015).

For the treatment of cough, opioid analgesic are the most effective antitussive drugs in clinical use (Rang et al., 2014) among which, the most currently and frequently used medicines in clinical practices are the centrally acting agents as synthetic drugs like Codeine, Codeine phosphate, Destrometorphan, Noscapine, Pholcodeine, etc. (Rang et al., 2014) used alone or in combinaition, prescribed as opioids mainly associated with various side effects such as nausea, vomiting, constipation, drowsinesss, confudion, dry mouth, dizziness, seating, facial flushing, headache, vertigo, bradicardie, tachycardia, palpitation, orthostatic hypotension, hypothermia, etc. (Sweetman, 2002) and are mainly used to suppress dry, painfuls, annoying and delibilating cough medulla, thereby raising cough threshold (Ashutosh et al., (2012); (Paneliya et al., 2015; Khawas et al., 2018; Petros, 2020).

However, the effectiveness of used antitussive and expectorant synthetic drugs in Western medicine has been challenged recently, and in general, it remains unsatisfactory in the treatment of cough, questionable clinical benefits despite being the widely used drugs in the world (Dicpinigaitis et al., 2014; Petros, 2020). In addition, adverse effects like constipation, drowsiness, respiratory depression, decreased expectoration, hypotension, addictive liability and hallucination occurring in large doses, among already other cited above, limit their therapeutic benefits in humain (Saraswathy et al., 2014; Petros, 2020).

Hence, currently, there is a serious and urgent need for the development of safe, effective antitussive and expectorant therapeutic drugs in the treating of persistent coughs as alternative to existing synthetic antitussive and expectorant drugs as suggested or preconised by Song et al., (2015). Herbal medicine or medicinal plants contain various bioactive ingredients or components belonging to different phytochemical groups and have got growing attention as potential therapeutic agents to prevent and treat cough due to their efficacy and low risk adverse effects (Zhou et al., 2013; Sararwathy et al., 2014; Petros, 2020). Thus, herbal therapy may be effectively used for the treatment of mild to persistent cases of coughs with sometimes fewer side effects than conventional synthetic drugs (Lin et al., 2016; Petros et al., 2020).

*Securidaca longepedunculata* (synonymes: *Elsota longipenduculata* Kuntze, *Lyphostyles oblongifolia*, *Lophostylis angustifolia* Hochst. Lophostylis oblongifolia Hochst. Securidaca angustifolia Miq. Securidaca longepedunculata var. angustifolia Robyns Securidaca longepedunculata var. parvifolia Oliv. Securidaca oblongifolia Benth. & Hook. f. ex Sond. Securidacaspinosa Sim (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:692714-1, 2023).

The violet tree is the most popular of all the traditional medicinal plants in South Africa and is used for almost every conceivable ailment. The roots are extremely poisonous, smell like wintergreen oil and contain methyl salicylate which may partly indicate why they have a

wide diversity of uses, such as arrow poison in some parts of Africa including West Africa. The roots and bark are taken orally either powdered or as infusions for treating chest complaints, headache, inflammation, abortion, ritual suicide, tuberculosis, infertility problems, venereal diseases and for constipation. Toothache can also be relieved by chewing the roots. Mixed roots of the violet tree and dwarf custard apple are used to treat gonorrhea. Powdered roots or wood scrapings are used to treat headache by rubbing them on the forehead, while infusions from the roots are used to wash tropical ulcers. In Limpopo, the vhaVenda people use roots for mental disorders and as protection against children's illness during breastfeeding. It is also believed that many African people use the powdered violet tree roots as a sexual boost for men. The vhaVenda people mix the powdered root with *mageu* (maize or sorghum beverage) and it is given to a man to drink if he is sexually weak.

The bark is used to make soap, fibre for fishing nets, baskets and strong threads that are used to sew bark cloth. In Zimbabwe, the roots are used to treat people who are believed to be possessed by evil spirits, for snakebite as well as for coughs when pounded with water and salt (https://www.google.com/search?q=securidaca+longepedunculata&oq=&gs_lcrp=EgZjaHJvbWUqCQgBECMYJxjqAjIJCAAQIxgnGOoCMgkIARAjGCcY6gIyCQgCECMYJxjqAjIJCAMQIxgnGOoCMgkIBBAjGCcY6gIyCQgFECMYJxjqAjIJCAYQIxgnGOoCMgkIBxAjGCcY6gLSAQk0NDU5ajBqMTWoAgiwAgE&sourceid=chrome&ie=UTF-8n, 2023;https://tropical.theferns.info/viewtropical.php?id=Securidaca+longipedunculata,2023; https://www.worldfloraonline.org/taxon/wfo-0000503535, 2023).

Informations from some traditional patricians in Democratic Republic of Congo, several have revealed that aqueous decoction of the roots to treat cough, diarrhea, dysentery, rheumatism; abodominal and dorsal pains, and divers infections.

Medicinal - Violet tree is a most popular traditional medicinal plant in many African countries. Powdered roots and barks in infusions are traditionally used to treat headache, stomach and chest problems, inflammation, tuberculosis, venereal diseases, constipation, toothache among others. It is reported to have antimicrobial activities against protozoa, bacteria and fungi. The active compound securinine has an activity against malaria causative agent Plasmodium falciparum. Xanthone compounds from the root bark confer action against erectile dysfunction.

Soap-The bark is used for soap, Fibre-The strong and durable fibres from the inner bark are used for fishing nets, baskets, bark cloth and strong threads, Food – Young leaves are used as vegetables and in sauces. Root infusions with maize or sorghum used as a beverage, Fodder – Animals feed on the roots, Apiculture-The flowers are frequented by bees for honey production. Seedss are used in cosmetic preparations, after pounding and short cooking. The extracted oil serve for skin care and hairs. This oil is reputed toxic in the case of digestion https://options.nri.org/background/plants-database/securidaca-longepedunculata, 2023).

The roots of S. longepedunculata serve in different preparations in Africain traditional medicine. Its essential oil produced from roots contains more than 90% methy salicylate. The medicinal applications of the root are principaly the treatment of teethpains, eczema, dermitis allergics, prurits and anemia (https://fr.wikipedia.org/wiki/Securidata-longepedunculata, 2023).

Roots are used in the treatment of rhumatism, bite snakes, poisoning, swelling pains, meningitis, achings, itchings, teethpains and leprosy. They are also exploited for the treating of constipation, cough, diarrhea, dysentery, fever, head pains, vaginal itchings, malaria and pneumonia. The stem barks are used to treat filariosis, malaria, bite snakes. The leaves are effective against constipation, urinary retention, conjonctivitis and cataracts. The branch stems are fibrous and serve to the fabrication of pieces of string. The leaves and stem bark, and particularly roots have ammoniac odour or methyl salicylate, they contain a deadly or hlttps://www.vetmeduni.ac.at/fileadmin/v/antimalaria-plants-bf/Fiche_Securidaca_longepedunculata.pdf); https://fr.wikipedia.org/wiki/Securidaca_longipedunculat alethal poison. (https://www.worldfloraonline.org/taxon/wfo-0000503535, 2023.

From chemical composition, the plant contains senegenine modifiable in magnesium senegenate obtained by saponin hydrolyse (fonctions acides salifiables par le magnésium), and its presursor soluble flavonoid presenegenine, essential oil to methyl salicylate more than 90% (https://www.wikiphyto.org/wiki/securidaca, 2023).

Some biological activities of *S. longependuclata* were evaluated and reported. They include the effects of *Securidata longepedunculata* on ionic currents and contraction on cultured rat skeletal muscle cells (Mouzou et al., 1999), trypanocidal activity of extracts (Atawodi et al., 2003, 2005; Aderbauer et al, 2008; Nibret at al, 2010; Setzer et al., 2012, Tauched, 2014), antitrypasomal and haemtological effects of selected Nigerian medicinal plants in Wistar rats (Abubakar et al., 2005), effect of *Securidata longepedunculata* (Polygalaceae) extract on intracellular calcium transient in cultured skeletal muscle cells (Mouzou et al., 2007), *in vivo* trypanocidal effect of aqueous extract of *Securidata longepedunculata* and its phytochemical analysis (Haruna et al., 2013), anti-inflammatory and analgesic activities of leaf and stem bark methanol extract (Afale et al., 2014), evaluation of

antitrypanosomal effect of stem bark from *Securita longependuculta* against *Trypanosoma brucei brucei* in infections in Wistar rats (Taucheed, 2014), antityrypanosomal activity of the methanol whole root extract (Eke, 2015), anti-inflammatory activity and toxicological effect of root bark in albino rats (Gbadamosi et al., 2017), safety assessment, *in-vivo* anti-trypanososomal activity of methanol root extract of *Securidata longepedunculata* in mice infected with *Trypanosoma brucei brucei* Haruna and Elige (2016), phytochemical screening and antimicrobial efficacy of the root bark extracts (Adejunon et al., 2019), *in vivo* antimalarial activity, toxicity and phytochemical composition of total extracts from *Securidata longepedunculata* Fresen (Nguta and Mwanzia, 2010), total triterpene content, antioxidant activity and acute toxicity study (Karana et al;, 2022), LCMS/MS analysis of the ethyl acetate extract of *Securidata longepedunculata* Fresen (Polygalaceae) stem bark (Abubakar et al., 2022), anthelmintic activity of *S. longepedunculata* (adieli et al., 2023), the insecticidal activity of extracts from organs of *s. longepedunculata* (guiré et al., 2024), the *in vitro* regeneration of the seeds of *s. longepedunculata* (mulumbati et al., 2023), the inhibitory activities of two compounds from *securidata longepedunculata* Fresen on acethylcholinesterase from wheat pest *Shizaptis graminum* Rondani (Guiré et al., 2024).

Nowadays, several medicinal plants claimed to cure different coughs in traditional medicine worldwide during daily practioners practices, are scientifically studied to prove in animal model their claimed antitussive and expectorant effects. Many reported results on medicinal plant extracts in this field have revealed that these natural materials are endowed with these both biological activity expressed at different extents (Li et al., 2010; Guo et al., 2016; Hernandez et al.; 2018; Khawas et al., 2018; Wu et al., 2018; Petros, 2020; Bilah et al; 2021; Bedse et al., 2023).

The aim of the present study is to evaluate the antitussive and expectorant activities of aqueous and methanol 80% extracts, soluble fractions like chloroform, ethylacetate, *n*-butanol and residual aqueous and crude polysaccharides, and acute and subacute toxicity of aqueous extract from *Securidaca longepedunculata* roots in animal model since until now, there is no study reported on this medicinal plant part in this field and the present study is carried out for the first time.

## 2. MATERIALS AND METHODS

**2.1 Plant materiel:** Fresh roots of *Securidaca longepedunculata* Fresen. (Polygalaceae) were collected in Kinshasa, capital of Democratic Republic of Congo (DRCongo) in September 2023. The plant was identified in Institut National d'Etudes et de Recherches Agronomiques (INERA), Department of Biology, Faculty of Sciences and Technology of University of Kinshasa. A voucher specimen of the plant was deposited in the herbarium of this institute and another in the laboratory of Pharmacognosy and Phytochemistry of the faculty of Pharmaceutical Sciences of the same university.

**Figure 1: *Securidata longepedunculata*, leaves, twigs, flowers and fruits.**

(https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:692714-1, 2023). The plant material was dried at room temperature for one week and the resulting dried material was reduced to powder in traditional mortar. The resulting powder was kept ed in an brown bottle hermatically closed before use.

**2.2. Preparation of extracts and fractionation:** 50 g of dried roots powdered were boiled with 300 ml distilled water on a hotplate at $100^{o}$ C for 15 minutes. After cooling and filtration on cotton and filter paper F1001 grade (CHMLAB GROUP, Barcelona, Spain), the resulting filtrate was evaporated *in vaccuo* giving dried extract denoted as SLAR-1 (33.35). An amount of 10 g of SLAR-1 extract was dissolved 100 ml distilled water and iltered as described above and the resulting filtrat was successively and exhaustively extracted with solvents with different polarities as chloroform, ethylacetate, *n*-butanol. All fractions including residual aqueous phase were treated as described above giving corresponding died extracts denoted as :SLAR-1.1 (2.05g), SLAR-1.2 (2. 46 g), SLAR-1.3 (1.85 g) and SLAR-1.4 (2.54 g), corresponding to soluble fractions chloroform rich in steroids and terpenoids, ethylacetate rich in flavonoids, *n*-butanol rich in saponins and residual aqueous rich in other phenolic compounds than

flavonoids. Next, 20 g other plant material were macerate with 80% methanol for 24 h. After filtration giving a macerate, the marc was exhaustively percolated with the some solvent giving a percolate. Macerate and percolate were combined and evoporated to rotative evaporator yielding a dried extract denoted SLAR-2 (14.16 g). (Cimanga et al., 2015). SLAR: *Securidaca longependunculta* roots

**2.3. Extraction of crude polysaccharides:** 200 of powdered leaves were macerated with 300 ml distilled water for 24 h. After filtration on filter Whatman N°1, the filtrate was concentrated *in vaccuo* to 30 ml. To this concentrated aqueous solution, 100 ml of ethanol 95°C was added and left in freezer at 4°C for 48 h. In these conditions a white precipitate was obtained, washed with ethanol and dried in etuve at 50°C for one day giving a dried extract of crude polysaccharides (16.34 g). Tested with phenol/$H_2SO_4$ conc., it showed a colour as a positive test for the presence of polysaccharides (Soniamol et al., (2011).

**2.4. Phytochemical screening:** It was carried out by using tube solutions and thyn layer chromatography (TLC) with appropriate chemical reagents and different mobile phases described in the literature (Harborne, 1998).

**2.5 Evaluation of antitussive activity against ammonia induced cough:** Antitussive effects of aqueous SLAR-1 and methanol 80% SLAR-2, and soluble fractions chloroform SLAR-1.1, ethylacetate SLAR-1.2, *n*-butanol SLAR-3. and residual aqueous SLAR-1.4 from *Securidaca longepedunculata* roots were evaluated using different classical models causing cough among which ammonia hydroxide liquor induced cough in Wistar rats according to the reported method of Wang et al.., (2012), Liu et al., (2015), Guo et al., (2016) and Uwaya et al., (2023). Each rat was placed in a dessicator and exposed to 25% $NH_4OH$ soaked in a piece of cotton wool or ball for 2 min followed by the oral administration of the test extracts et soluble fractions from *Secutidata longepen dunculata* roots for 60 min. During the amononia exposure, the rats were taken out from the dessicator and placed in a conservation chamber for counting of bouts of coughs (cough frequencies) produced within 15 minutes and were continuously monitorited by a trained observer. The cough was detected as a contraction of thoracic and abdominal muscles followed by the mouth opening with a coughing sound and jerking of the front body of each treated rat (Hernandez et al., 2018). The rats and the number of couch frequencies were recorded after 15 min. Rats with couching 5-20 times in 15 minutes were selected as eligible rats for the experiment. After 24 of recovery, they were divided into different groups (3 rats for each oral dose) and treated orally fort three consecutive days (from 9-10 AM daily). As follows:

- Group I as negative control was treated orally with 3% Tween 80 and ammonia hydroxide solution,
- Group II was given orally 10 mg codeine phosphate body weight (bw),
- Group III received orally of aqueous extract SLAR-1,
- Groups IV to VII were orally administered soluble fractions SLAR-1.1 to -1.4,
- Group VIII was orally administered methanol 80% extract SLAR-2,
- Group IX was given orally the same dose of crude polysaccharide CPSLAR.

All treated rats were orally administered 100 and 200 mg/kg body weight (bw) of extracts, soluble fractions and crude polysaccharides for three consecutive days.

Half an hour after oral administration of the last treatment dose, each rat was placed in the dessicator with 0.3 ml 25% amomonium hydroxide soaked in a piece cotton ball for 15 minutes as already mentioned above. Then, the rats were taken out, the latent period of cough and the cough frequencies produced within 15 minutes were counted and recorded. All rats were monitorited continously trained technician blinded to the treatment given and the number of cough frequencies and latency times to initial cough response were noted. The antitussive activity was assessed as the percentages of inhibition of the number of cough frequencies in terms of that in the control negative group calculated using the following formula:

$$\% \text{ Inhibition of cough frequencies} = \frac{NCFNC - NCFTS}{NCFNC} \times 100$$

Where NCFNC was the number of cough frequencies in negative control and NCFTS the number of cough frequencies of treated sample.

**2.6. Evaluation of antitussive activity against sulfur dioxide induced cough:** Antitussive activity of *S. longepedunculata* roots samples against sulfur dioxide induced cough in Wistar rats was assessed according to the method previously described by Paneliya et al., (2015); Guo et al., (2016) and Billah et al., (2021). A dessicator containing vial 2 ml at the base, holding a pipette from the top and floored with a wired gauge, was used in the experiment. At first, the vial contained 500 mg/ml sodium hydrogen sulfite ($NAHSO_3$) and 0.2 ml of sulphuric acid ($H_2SO_4$) were added using the mounted pipette where the following reaction took place:

$2NaHSO_3 + H_2SO_4 \rightarrow 2SO + Na_2SO_4 + H_2O$

Rats were placed in the wired platform in the dessicator after 15 seconds and group II of rats was subjected to the exposure to sulfur dioxide ($SO_2$) for 45 seconds as negative control. Theafter, rats were withdrawn from the dessicator and paced in observation cages with an open-ended funnel filter to which a stethoscope attached to count the reflex of cough for 5 minutes in contact of sulfur dioxide. The experiment was repeated for all groups at 30, 60, 90 and 120 minutes after oral drug treatment. The cough frequencies were recorded at the end of the experiment. The percentage inhibitions of the

number of cough frequencies were calculated according the formula above.

**2.7. Evaluation of antitussive activity against citric acid induced cough:** Antitussive effects of extracts and soluble fractions from *S. longepedunculata* against citric acid induced cough in Wistar rats were evaluated according to the method previously described by Zang et al., (2009) and Guo et al., (2016). After 24 h recovery, the selected rats were randomdly divided into 9 groups as followed.

- Group I received orally 3% Tween 80 as negative control,
- Group II received 10 mg codeine phosphate as positive control,
- Group III was orally administered aqueous extract SLAR-1,
- Groups IV to VII were orally administered soluble fractions SLAR-1.1 to-1.4,
- Group VIII was orally given methanol 80% extract SLAR-2
- -Group IX was orally administered the same doses of crude polysaccharide CPSLAR.

All treated rats were orally administered 100 and 200 mg/kg body weight (bw) of extracts, soluble fractions and crude polysaccharides for three consecutive days. Guinea-pigs were placed in a glass chamber to screen for sensitivity and 33% citric acid (W/V) was spayed for 2 minutes in groups II to IX. The cough frequencies and the latent period of cough were observed and guinea-pigs with the frequency of cough (10-30) were selected for further antitussive test. All rats were orally administered 100 and 200 mg of aqueous and methanol 80% extracts, soluble fractions and crude polysaccharides, and individually placed into transparent perpex airlight chamber. After 45 minutes, all rats were exposed to 1 M citric acid aerosols (1 ml/min, flow rate) for 30 seconds. The latent period of cough from the start to the onset of cough, and cough frequencies were observed for 5 minutes and recorded (Zang et al., 2009a,b; Guo et al., 2016). The percentage inhibitions of cough frequencies were calculated using the formula above.

**2.8. Antitussive activity against capsaicin induced cough:** The experiment against capsaicin induced cough in Wistar rats was performed according to the methods previously reported by Liu et al;, (2015) and Billah et al., (2021). A 500 ml glass beaker was used in this regard where the rats were individually placed and subjected to atomized spay of caspsaicin solution (100 µmol/L) for 10 seconds. The frequency of coughing was counted for 2 minutes. After recovery on 24 h, rats were orally administered the respective treatments (100 and 200 mg/kg bw) and re-exposed to capsaicin nebulization. Again, the bouts of coughing or cough frequencies were recorded.

**2.9. Expectorant activity assessment:** Phenol red secretion experiment were performed using the method previously described by Zhang et al., 2009a, b; Guo et al., 2016). To evaluate the expectorant activity of *S. longepedunculata* roots extracts, soluble fractions a and crude polysaccharides were tested at oral administered doses of 100 and 200 mg/kg bw. Each rat was treated with test samples for 30 minutes before injection of 5% phenol red physiologic saline solution (0.2 ml/20 g). After 30 minutes, all rats were sacrified by cervical dislocation without damaging the trachea. The trachea was removed between the thyroid cartilage and the main stem bronchi and placed into 2 ml normal saline. After ultrasonification for 10 minutes, 0.1 ml of 1 M NaOH solution was added. A spectrophotometer was used to measure the optical density of the mixture at 546 nm.

In all antitussive and expectorant tests, all biological activities were evaluated in treated rats for a maximum administration doses of 3 days.

**2.10. Acute and subacute toxicity of aqueous SLAR-1 extract of *S. longepedunculata* roots**

**2.10.1. Acute toxicity:** The acute toxicity of the aqueous extract of *S. longepedunculata* roots was evaluated in Wistar rats according to the procedure described by the Organization for Economic Cooperation and Development (OECD) guideline for testing chemicals, TG420 (OECD, 2001). Animals (body weight: 140– 150 g, aged 8–10 weeks, of either sex) were divided into two groups.

- Group I (5 rats) orally received 5 ml distilled water body weight (bw) and constituted the negative control group.
- Groups II (5 rats each) received once orally single oral dose of 5000 mg/kg bw of the aqueous extract. The animals were observed for toxic symptoms continuously for the first 4 h dosing and were daily weighed. Finally, all animals were then maintained in daily observation and the number of toxic effects, survivor and mortality were recorded for further 28 days (OECD, 2001; Ogbonnia et al., 2008; Kripa et al., 2011; Gandliare et al., 2013).

**2.10.2**. **Subacute toxicity:** The sub-acute toxicity of the aqueous extract of *S. longepedunculata* roots was evaluated according to the procedure described by Kripa et al. (2011) and Gandliare et al. (2013). Briefly, Wistar rats were used and divided into four groups.

- Group I (2 rats) orally received daily normal saline solution (NaCl 0.9%) and constitute the negative control.
- Groups II, III and IV (5 rats for each oral dose) orally received daily 500, 1000 and 5000 mg/kg bw of aqueous extract each day for 28 days. The body weight was daily recorded each week. Animals were observed daily for toxic symptoms, behavior, alteration, digestive troubles, food and water intake, etc. and for survivor and the occurring of mortality during 28 days period of the experiment.

**2.11. Analysis of biochemical and hematological parameters:** Blood from rats having received 5000 mg/kg bw in acute toxicity test of aqueous extract SLAR-1 was collected from tail vein on Day 28 for analysis. For biochemical parameters, blood was centrifuged at 4000g for 5 min to obtain plasma, which was stored at -20°C: glucose, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), uric acid, total cholesterol, triglycerides, high–density lipoproteins (HDL), low–density lipoproteins (LDL), total and direct bilirubin were quantified using Architect (Abottâ) automation with Boehringen Ingelheimâ biochemical kits. Total proteins were estimated using Biuret's method (Saha et al., 2011). Hematological parameters analysis was carried out using an automatic hematological analyzer (Coulter STK, Beckam) with appropriated kits. The differential leucocyte count was performed with an optical microscopy after staining and, in each case, 100 cells were counted (Dacie and Lewis, 1991; Akpanabiatu et al., 2013)

For mineral elements, 10 ml of blood of animals having received 5000 mg/kg bw of aqueous extract was collected and incinerated at 450°C for 24 h in a mufleand acid digest. The material for analysis was prepared by oxidizing sample with nitric/perchloric acids 2:1. The levels of minerals were determined with flame atomic absorption spectrophotometer (Perkin-Elmer2880 Model) and the inorganic phosphourus was estimated by phosphomolybdovanate method (AOAC, 1990).

**2.12. Histopathological study:** Histopatological study of vital organs such as heart, kidney, liver, spleen, large intestine and lungs was carried out according to the procedure previously described by Lamb (1981). The organ pieces (5-8 µm) were fixed in 10% formalin for 24 h and washed in running distilled water for against 24h. After dehydratation in an autotechnicon, the cleared organs were embedded by passing through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtone and the slides were stained with haemtoxyllin-eosin and observed under electronic microscopic. The dried organs were weighted.

## 2. RESULTS AND DISCUSSION

**3.1 Phytochemical screening:** This study revealed the presence of alkaloids, phenolic compounds such as, flavonoids, tannins, anthraquinones, terpenoids, steroids, saponins, polysaccharides and reducing sugars. Our results were in good agreement with Haruna et al., (2013; Adeujun at al., 2019 and https://www.wikiphyto.org/wiki/securidaca, 2023). Anthocyanins and cardiotonic heterosides were not detected in our experimental conditions.

**Table 1: Phytochemical screening**

| Chemical groups | Results | Chemical groups | Results |
|---|---|---|---|
| Alkaloids | + | Phenolic compounds | + |
| Antocyanins | - | Polysaccharides | + |
| Anthraquinones | _ | Tannins | + |
| Cardiotonic heterosides | + | Reducing sugars | + |
| Coumarines | _ | Saponins | + |
| Flavonoids | _ | Steroids | + |
| Glycosides | + | Terpenoids | + |

**3.1. Antitussive activity of extracts, soluble fractions and crude polysaccahrides from *S. longepedunculata* roots**

**3.1.1. Antitussive activity of Codeine phosphate, extracts, soluble fractions against ammonium hydroxide liquor**

The effects of Codeine phosphate used as standard antitussive drug, aqueous extract SLAR-1 and 80% methanol SLAR-2, soluble fractions SLAR-1.1 to SLAR-1.4 and crude polysassharides CPLAR from *S. longepedunculata* roots against ammonia hydroxide liquor induced cough were shown in Table.1. All tested samples significantly increased latent period of cough and decreased cough frequencies (cough bouts) in dose-dependent manner compared to negative control with significant difference was observed ($p < 0.05$) (Table 1).

Ammonium hydroxide liquor induced cough model was widely used for the evaluating antitussive active activity of extracts, soluble fractions and isolated compounds form medicinal plants as well as synthetic substances. The cough frequencies and the cough incubation period were common indices for usage of this framework.

In the present study, the latent period of cough and cough frequencies were remarkably increased and reduced respectively in treated groups with aqueous SLAR-1, 80% methanol SLAR-2 extracts, soluble fractions SLAR-1.1 and 1.4 as well as crude polysaccharides CPSLAR in dose-dependent manner (Table 1).

Tested at the high oral dose of 200 mg/kg bw, aqueous SLAR-1 and methanol SLAR-2 extracts delayed latent period of cough by 4.34±0.04 and 1.50±0.02% respectively, as also reported by Petros (2020) for methanol 80% extract of *Adthoda shiperianta* (Hoscht.) (Acanthaceae) and produced significant reduction of number of cough frequencies or cough bouts to 6.56±0.02 and 5.65±0.04 for an inhibition of 90.02±0.01 and 91.71±0.00% while Codeine phosphate and crude polysaccharides CPLAR showed 6.56±0.03 and 6.83±0.06.

**Table 1: Effects of Codeine phosphate, extracts and soluble fractions on ammonium hydroxide induced cough.**

| Groups | TTT (mg/kg bw) | LPC (seconds) | % PILPC | NCF | %INCF |
|---|---|---|---|---|---|
| CN | 3% Tween 80 | 77.05±0.07 | 0.00±0.00 | 65.75±0.07 | 0.00±0.00 |
| CP | 10 | 190.50±0.04 | 147.24±0.01 | 6.56±0.03 | 90.02±0.03 |
| SLAR-1 | 100 | 110.55±0.03 | 4.15±0.03 | 6.15±0.04 | 90.64±0.04 |
| | 200 | 115.54±0.05 | 4.34±0.04 | 6.56±0.02 | 90.02±0.01 |
| SLAR-2 | 100 | 115.35±0.03 | 2.07±0.03 | 5.45±0.02 | 91.71±0.00 |
| | 200 | 118.33±0.06 | 1.50±0.02 | 5.65±0.04 | 91.40±0.04 |
| SLAR-1.1 | 100 | 112.35±0.04 | 47.03±0.04 | 11.54±0.04 | 82.44±0.06 |
| | 200 | 114.63±0.03 | 43.58±0.02 | 13.05±0.02 | 80.15±0.04 |
| SLAR-1.2 | 100 | 90.25±006 | 1.71±0.03 | 7.71±0.05 | 88.27±0.00 |
| | 200 | 105.62±0.04 | 23.76±0.05 | 8.24±0.03 | 87.46±0.03 |
| SLAR-1.3 | 100 | 105.60±0.05 | 37.05±0.04 | 14.65±0.03 | 77.71±0.05 |
| | 200 | 108.12±0.03 | 32.53±0.05 | 16.04±0.00 | 75.60±0.03 |
| SLAR-1.4 | 100 | 100.36±0.02 | 30.25±0.02 | 7.87±0.04 | 88.03±0.06 |
| | 200 | 106.25±0.00 | 37.89±0.00 | 8.43±0.06 | 87.17±0.04 |
| CPSLAR | 100 | 112.87±0.04 | 46.48±0.03 | 6.35±0.04 | 90.03±0.02 |
| | 200 | 115.35±0.06 | 49.70±0.00 | 6.83±0.06 | 89.61±0.05 |

NC: negative control, CP: Codeine phosphate, TTT: treatment, LPC: latent period of cough, %PILPC: percentage increase of latent period of cough, NCF: number of cough frequencies, %INCF: percentage inhibition of the number of cough frequencies, SLAR-1: aqueous extract, SLAR-2: 80% methanol extract, SLAR-1.1 to 1.4: chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions from the partition of aqueous SLAR-1 extract.

reduction frequencies for percentage inhibitions by 90.02±0.0 and 89.61±0.05% and latent period of cough of 198.50±0.04 and 115.35±0.06 at administered oral doses of 10 and 200 mg/kg bw respectively.

Codeine phosphate, aqueous SLAR-1 and 80% methanol SLAR-2 extracts, and polysaccharides seemed to have similar antitussive activity since no significant difference was not deduced ($p > 0.05$).

At the highest oral dose of 200 mg/kg bw, soluble fractions SLAR-1.1 to -1;'also acted by increasing latent period of cough from 105.62±0.04 to 114.63±0.03 to 106.25±0.06 (Table 1) with its percentage increases from 23.76±0.05 to 43.58±0.02% (Table1) and significantly reduced the number of cough frequencies form 8.24±0.03 to 16.04±0.00 (Table 1) causing its inhibition production from 87.46±0.03 to 75.01±0.03%, all compared to negative control showing latent period of cough of 77.05±0.05 and cough frequencies of 65.75±0.05.

Etlylacatate SLAR-1.2 showed high antitussive activity compared to remaining soluble fractions SALR-1.1, 1.3 and 1.4 (Table 1). Crude polysaccharides CPSLAR produced latent period of cough of 115.35 seconds and increase percentage of this parameter to 49.70±0.00% at oral dose of 200 mg/kg bw, with the number of cough frequencies of 6.83±0.06 for its inhibition of 89.61±0.05%. CPSLAR showed weak and high antitussive activity compared aqueous SLAR-1 and 80% methanol SLAR-2 extracts and soluble fractions SLAR-1.1 to -1.4 respectively, with significant difference ($p < 0.05$) (Table 1). Our results were only qualitatively in good agreement with Guo et al., (2016) and Petros et al., (2020).

Figure 1 showed the variation of latent period of cough caused by Codeine phosphate (CP), aqueous SLAR-1 and methanol SLAR-2 extracts, and soluble fractions SLAR-1.4 to 1.4 tested at 10, 100 and 200 mg/kg bw respectively. Aqueous SLAR-1, 80% methanol SLAR-2 extracts and soluble fractions SLAR-1.1 to -1.4 prolonged latent period of cough incubation times in treated rats in dose-dependent manner. (Fig. 1).

At the administered the highest oral dose of 200 mg/kg bw, aqueous SLAR-1and 80% methanol SLAR-2 produced latent period of cough (LPC) after 115.54 and 118.33 seconds for their increase percentage by 4.34±0.04 and 1.50±0.02%. The longer cough incubation period and decrease coughing frequencies showed stronger antitussive effects of the both tested extracts on relieving cough and exhibited thus, stronger antitussive effects (Fig. 1). The increase cough latent period of cough from 107.07 to 113.56 seconds for their increase percentage by 32.53±0.05 and 43.58±0.02% and decrease cough frequencies from 11.33±0.03 and 17.62±0.03 for their inhibition percentage by 75.72±0.05 and 73.11±0.04%, compared to negative control with LPC of 77.05±0.07 and NCF of 65.75±0.07, showed by the potential effects of the tested soluble chloroform, ethylacetate, *n*-butanol and residual aqueous SLAR-1.1 to -1.4 respectively at the same highest oral dose on delaying cough production was considered as a manifestation of their important reduction of cough in treated rats (Fig. 1).

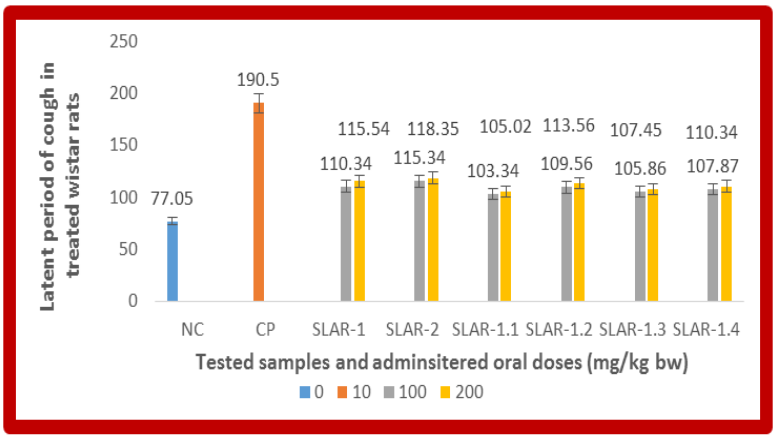



**Figure 1: Latent period of cough produced by negative control (CN), Codeine phosphate (CP), aqueous SLAR-1, 80% methanol SLAR-2 and soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous SLAR-1.1 to -1.4 respectively at 10, 100 and 200 mg/kg bw respectively in ammonium hydroxide induced coughing compared to negative control (CN).**

$NH_4OH$-induced cough model used in the present study, was a currently and commonly used chemical stimuli model with relatively easy procedure for evaluation antitussive actions of medicinal plant extracts, soluble fractions, pure isolated compounds form medicinal plants and synthetic substances. This model was a valid method employed previously by several investigators (Wang et al., 2012; Liu et al., 2015; Hernandez et al., 2018; Kuang at al., 2018; Wu et al., 2018; Petros, 2020) to evaluate antitussive activity of medicinal extracts.

Therefore, in the present study, the antitussive effects of extracts, soluble fractions and crude polysaccharides from *S. longepedunculata* roots, were demonstrated in the *vivo* experiment by extending the incubation times and reducing coughing frequencies in ammonium hydroxide-induced cough in animal model (Fig. 1).

In our present study, aqueous SLAR-1 and 80% methanol SLAR-2 extracts, and soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous SLAR-1.1 to-1.4 respectively from *S. longepedunculata* were found to be able to prolong cough time and decrease remarkably coughing frequencies in treated rats in dose-dependent manner (Fig. 1). The cough incubation period exhibited the potential effects of the drug on delaying cough. The longer cough incubation period showed stronger antitussive effects of extracts, soluble fractions and crude polysaccharides form *S. longepedunculata* roots on relieving cough. For the antitussive activity of crude polysaccharides reported in the presents study, our results were in good agreement with Sutovska et al., (2009, 2011); Nosalova et al., (2013); Liu and Lai (2016) and Raja et al., (2023) who had previously reported the antitussive activity of polysaccahrides from other medicinal plants.

Figure 2 showed the change of cough frequencies induced by Codeine phosphate (CP), aqueous SLAR-1 and methanol 80% SLAR-2 extracts, and soluble fractions SLAR-1.1 to 1.4 in amononia liquor induced cough. It was observed that number of frequencies cough induced by administered aqueous SLAR-1, 80% methanol SLAR-2 extracts and soluble fractions SLPAR-1.1 to -1.4 significantly decreased comparatively to negative control indicating that they exerted antitussive activity in considering the levels of their cough frequencies production and the reduction of the number of cough frequencies (NCF) reflected the potential effects of the tested extracts on delaying cough (Fig. 2).

Administered at doses oral of 100 and 200 mg/g bw respectively, aqueous extract SLAR-1 (CF = 20.36 and 22.02 respectively) exerted high antitussive activity compared to 80% methanol SLAR-2 extract (CF = 22.30 and 24.06 respectively) while soluble fractions SLAR-1.1 to -1.4 showed number of cough frequencies from 23.76 to 24.43 at administered oral dose of 100 mg/kg bw. This cough frequency significantly increase with the administration of the highest oral dose of 200 mg/kg bw taking back them to values from 25.03 to 26.42 (Fig. 2).

The cough was significantly reduced in the treated groups with soluble fractions SLAR-1.1 to 1.4 given either treated extracts SLAR-1 and -2 causing significant antitussive effect which may be related to the presence of different active constituents in aqueous SLAR-1 and 80% methanol SLAR-2 extracts and soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous SLAR-1.1 to 1.4 respectively. SLAR-1.1, 1.2 and 1.4 showing CF values ranging from 24.34 to 25.34 and 22.67 to 26.54 respectively (Fig. 2).

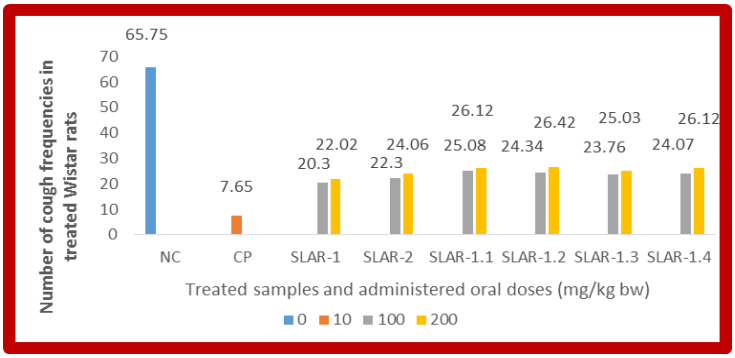



**Figure 2: Variation of the number of cough frequencies produced by negative control (CN), Codeine phosphate (CP), aqueous SLAR-1 and methanol 80% SLAR-2 extracts, and soluble fractions SLAR-1.1 to 1.4 from *S. longepedunculata* roots observed in amononium hydroxide liquor induced cough.**

**3.1.2. Antitussive activity of Codeine phosphate, extracts, soluble fractions against sulfur dioxide:** The results *of S. longepedunculata* roots extracts SLAR-1 and-2, and soluble fractions SLAR-1.1 to 1.4, in sulfur dioxide induced cough test were shown in Table 2. Codeine phosphate used as standard antitussive drug at 10 mg/kg bw, significantly suppressed the cough frequencies by 16.85±0.03 resulting in its inhibition of 69.82±0.02 % and exerted appreciable antitussive effect compared to aqueous SLAR-1, 80% methanol SLAR-2 extracts and soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous SLAR-1.1 to -1.4 respectively.

Pretreatment with aqueous SLAR-1 and methanol SLAR-2 extracts reached significant decrease of cough frequencies to 20.54±0.03 and 18.35±0.04 and caused its inhibition to 63.22±0.04 and 63.18±0.03% respectively at oral dose of 100 mg/kg bw and this effect remarkably increased with the use of high dose of 200 mg/kg bw (Table 2). Codeine phosphate (CP), aqueous SLAR-1 and methanol 80% SLAR-2 extracts on oral administration in treated rats at respective doses, induced significant decrease of cough frequencies in dose related See Table 1. Manner, thus, it could be assumed that CP and these all tested samples might be acting via the central system as also previously reported by Chimala and Deba, (2004) for *Cassia occidentalis* Linn. roots in the suppression of cough production in sulphur dioxide-induced couching.

**Table 2: Effects of *S. longepedunculata* extracts and soluble fractions, and codeine phosphate on sulfur dioxide-induced cough.**

| Groups | Treatment (mg/kg bw) | Number of cough frequencies | % Inhibition of cough frequencies |
|---|---|---|---|
| NC | 3%Tween 80 | 55.85±0.06 | 0.00±0.00 |
| CP | 10 | 16.85±0.03 | 69.82±0.02 |
| SLAR-1 | 100 | 20.54±0.0 | 63.22±0.04 |
| | 200 | 22.36±0.05 | 59.96±0.00 |
| SLAR-2 | 100 | 18.35±0.04 | 67.14±0.05 |
| | 200 | 20.56±0.06 | 63.18±0.03 |
| SLAR-1.1 | 100 | 23.48±0.05 | 57.95±0.02 |
| | 200 | 25.65±0.03 | 54.07±0.03 |
| SLAR-1.2 | 100 | 21.03±0.06 | 62.34±0.06 |
| | 200 | 22.06±0.04 | 60.50±0.04 |
| SLAR-1.3 | 100 | 25.15±0.03 | 53.00±0.04 |
| | 200 | 26.25±0.01 | 54.96±0.01 |
| SLAR-1.4 | 100 | 23.00±0.05 | 58.81±0.06 |
| | 200 | 24.05±0.06 | 56.93±0.03 |
| CSLPAR | 100 | 19.76±0.06 | 64.61±0.01 |
| | 200 | 17.07±0.04 | 69.43±0.02 |

Whereas, soluble fractions SLAR-1.4 to 1.4 reached also remarkable decrease of the cough frequencies to values from 21.03±0.06 to 25.15±0.03 causing its inhibition from 54.96±0.01 to 62.34±.05% respectively administered at the highest oral dose of 200 mg/kg bw, all compared to negative control producing 55.85±0.06 of cough frequencies. Aqueous SLAR-1 and methanol 80% SLAR-2 extracts, soluble fractions SLAR-1.1 to-1.4 as well as crude polysaccharides CPSLAR showed stunning ability to suppress sulphur dioxide induced-cough (Panliya et al., 2015;Khawas et al., (2018) after oral administration of all tested samples in dose-

dependent manner (Table 2). The significant decrease in the number cough frequencies (NCF) effort was evident at 30 minutes after treatment and the antitussive activity also significantly increase with the treatment times from 60 to 120 minutes. Suppression of cough production remained highly significant until the last measurements as also demonstrated by Khawas et al., 2018) for polysaccharides from *Psidium guayava* leaves. Our results were qualitatively in good agreement with Guo et al., (2016) and Khawas et al., (2018).

Crude polysaccharides CPSLAR produced also remarkable reduction of number cough frequencies in dose-dependent manner (Table 2) and administered at highest oral dose of 200 mg/kg bw, it showed NCF of 17.07±0.04 for an inhibition of 69.43±0.02% compared to negative control producing the number cough frequencies of 55.86±0.06.

Our results were only qualitatively in good accordance with Marina et al., (2008); Haq et al., (2013); Paneliya et al., (2015); Bhagyalakshmi et al;, (2016); and Guo et al., (2016).

Figure 3 showed the modification levels of the number cough frequencies engendered by Codeine phosphate (CP) and aqueous SLA-1 and 80% methanol SLAR-2 extracts in sulphur dioxide induced-coughing compared to negative control (NC).

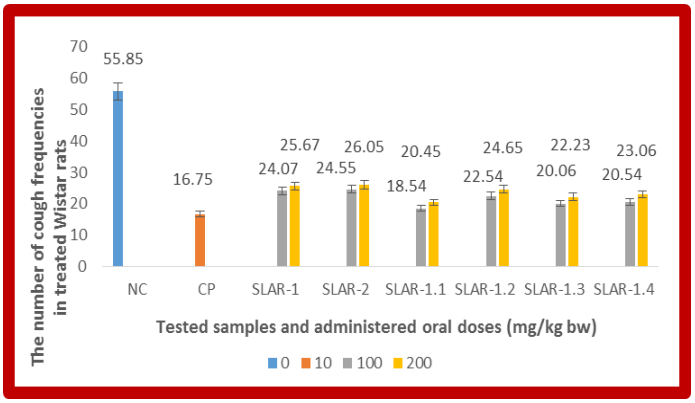



**Figure 3: Modification of number of cough frequencies produced by negative control (CN), Codeine phosphate (CP), aqueous SLAR-1, 80% methanol SLAR-2 extracts and soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous SLPAR-1.1 to -1.4 observed on sulfur dioxide ($SO_2$) induced cough.**

The number of the number of cough frequencies engendered by administered aqueous SLAR-1 and 80% methanol SLA-2 extracts were 20.07 and 24.55 at administered oral dose I00 mg/kg bw and increased progressively until values of 24.55 and 26. 05at 200 mg/kg bw respectively. Soluble fractions SLAR-1.1 to 1.4 produced CF from 18.54 22.54 and from 20.45 to 24.65 ( Fig. 3) administered at oral doses of 100 and 200 mg respectively, and significantly delayed in this way the cough production in treated rats. The reported number of cough frequencies (NFCF) in the present case were only qualitatively in accord with Guo et al., (2016).

**3.1.3. Antitussive activity of Codeine phosphate, extracts, soluble fractions against citric acid:** The results of *S. longepedunculata* roots aqueous SLAR-1, 80% methanol SLAR-2 extracts, soluble fractions chloroform, ethylacatate, *n*-butanol and residual aqueous SLAR-1.1 to -1.4 and Codeine phosphate on citric acid-induced cough test were presented in Table 3. Results revealed that negative control produced high latent period of cough (LPC) of 78.65±0.06 and cough frequencies of 14.12±0.08 while Codeine phosphate as standard antitussive drug significantly increased latent period of cough to 185.36±0.04 for a high percentage increase of 135.7±0.02%. It decreased the number of cough frequencies to 4.22±0.033 for an inhibition of 70.11±0.04% when negative control presented high number of cough frequencies of 14.12±0.08.

On the other hand, treatment with aqueous SLAR-1 and 80% methanol SLAR-2 extracts increase of latent period of cough in dose-dependent manner (Table 3). At the highest administered oral dose of 200 mg/kg bw, they increased this parameter to high values of 115.68±0.05 and 107.6±0.02 for their percentage increase of 47.08±0.05 and 36.50±0.06% respectively while negative control presented low value of latent period cough by 78.65±0.06. They produced significant decrease of the number of cough frequencies to 4.72±0.03 and 4.10±0.06 for an inhibition of cough frequencies of 66.57±0.04 and 70.96±0.04% respectively while negative control showed high cough frequencies of 14.12±0.08.

All soluble fractions SLAR-1.1 to -1.4 also displayed significant increase of LPC to values from 96.45±0.04 to 119.030.04 causing the percentage increases of this parameter to values from 35.19±0.03 to 59.56±0.05%, and showed significant decrease of CF from 5.71±0.03 to 11.43±0.02 for an percentage inhibitions from11.05±0.04 to 53.75±0.06% See Table 1, TTT: Treatment, LPC: latent period of cough, %ILPC: percentage increase of

latent period of cough, %INCF: percentage inhibition of the number of cough frequencies.

**Table 3: Effects of *S. longepedunculata* extracts and soluble fractions, and codeine phosphate on citric-acid-induced cough.**

| Groups | TTT (mg/kg bw) | LPC (seconds) | % ILPC | NCF (Number of cough frequencies) | % INCF |
|---|---|---|---|---|---|
| CN | 3% Tween 80 | 78.65±0.06 | 0.00±0.00 | 14.12±0.08 | 0.00±0.00 |
| CP | 10 | 185.36±0.04 | 135.7±0.02 | 4.22±0.03 | 70.11±0.04 |
| SLAR-1 | 100 | 98.63±0.4 | 25.42±0.03 | 5.65±0.05 | 59.98±0.02 |
| | 200 | 115.68±0.05 | 47.08±0.05 | 4.72±0.03 | 66.57±0.04 |
| SLAR-2 | 100 | 95.75±0.06 | 21.74±0.03 | 5.10±0.03 | 63.88±0.06 |
| | 200 | 107.36±0.02 | 36.50±0.06 | 4.10±0.06 | 70.96±0.04 |
| SLAR-1.1 | 100 | 115.11±0.04 | 46.35±0.04 | 10.36±0.04 | 26.62±0.00 |
| | 200 | 118.56±0.05 | 50.74±0.03 | 9.25±0.02 | 35.19±0.03 |
| SLAR-1.2 | 100 | 100.03±0.01 | 22.63±0.00 | 6.53±0.03 | 53.75±0.06 |
| | 200 | 112.45±0.04 | 27.18±0.06 | 5.71±0.03 | 59.56±0.05 |
| SLAR-1.3 | 100 | 117.36±0.03 | 49.21±0.04 | 13.25±0.04 | 6.16±0.02 |
| | 200 | 119.03±0.04 | 51.34±0.02 | 11.43±0.06 | 19.05±0.04 |
| SLAR-1.4 | 100 | 102.36±0.03 | 30.14±0.05 | 8.23±0.05 | 41.71±0.03 |
| | 200 | 106.54±0.05 | 32.91±0.01 | 7.03±0.02 | 50.21±0.04 |
| CPSLAR | 100 | 103.45±0.05 | 31.53±0.06 | 5.07±0.03 | 64.09±0.06 |
| | 200 | 108.80±0.03 | 38.33±0.04 | 4.32±0.00 | 69.40±0.04 |

Respectively all compared to negative control showing high LPC and of NCF of 65±0.06 and 14.12±0.08 respectively.

Our results were only qualitatively in good agreement with Fleskova et al., (2012); Nosalova et al., (2013a, b); Song et al., (2015); (Guo et al., (2016), Khawas et al., (2018) and Uwaya et al., (2023). Figure 4 showed the change of latent period of cough caused by the oral administration of Codeine phosphate (CP), aqueous SLAR-1 and 80% methanol SLAR-2 extracts, and soluble fractions SLAR-1.1 to 1.4 administered at oral doses of 10, and 100 and 200 mg/kg bw respectively in citric acid induced-cough compared to negative control (NC).

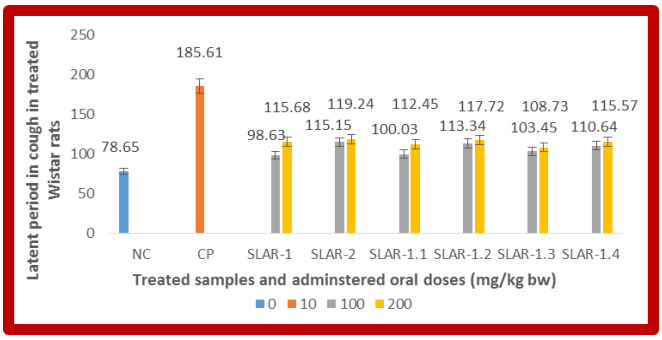



**Figure 4: Latent period of cough produced by negative control (CN), Codeine phosphate (P), aqueous SLAR- and 80% methanol SLAR-2, and soluble fractions SLAR-1.1 to 1.4 observed on citric acid ($C_6H_8O_7$) induced-coughing.**

Administered aqueous SLAR-1 and 80% methanol SLAR-2 extracts, and soluble fractions SLAR-1.1 to 1.4 at all oral doses, caused significant increase of latent period of cough in dose- dependent manner compared to negative control (Fig. 4). This finding was in good agreement with Guo et al., (2016).

Figure 5 indicated the variation of the number of cough frequencies of Codeine phosphate (CP), aqueous SLAR-1 and 80% methanol SLAR-2, and soluble fractions SLAR-1.1 to 1.4 administered at 10, 100 and 200 mg/kg bw respectively, showing decrease of the number of cough frequencies in dose-dependent manner in citric acid induced-cough comparatively to negative control (Fig. 5).

Aqueous SLAR-1 and 80% methanol SLAR-2, and soluble fractions SLA-1.1 to1.4 delayed significantly the production of cough since they reduced considerably the number of cough frequencies compared to negative control ($p < 0.05$) and exerted antitussive activity. Tested at 100 and 200 mg/kg bw respectively, 80% methanol SLAR-2 showed high antitussive activity (NCF = 4.10 and 5.10 respectively) compared to aqueous SLAR-1 extract (CF = 4.70 et 5.68 respectively), and soluble fractions chloroform, ethylacetate, *n*-butanol and residual

aqueous SLAR-1.1 to -1.4 (NCF from 8.23 to 13.25 and 7.03 to 11.43 respectively) The reported values for latent period of cough and the number of cough frequencies in the present study were in same ranges compared to those reported by Guo et al., (2016).

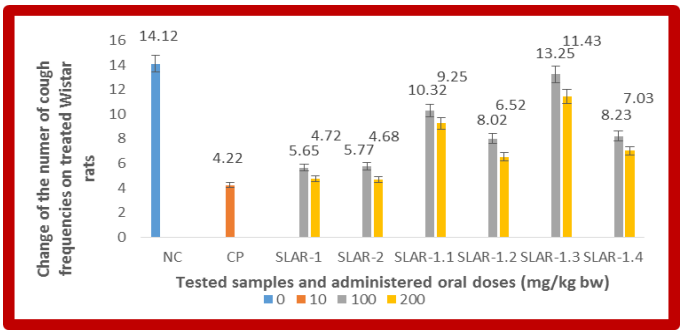
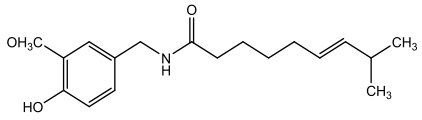



**Figure 5: Variation of cough frequencies of produced by negative control (CN), Codeine phosphate (CP), aqueous SLAR-1 and 80% methanol SLAR-2 extracts, and soluble fractions -1.1 to 1.4 observed in citric acid ($C_6H_8O_7$) induced-coughing.**

### 3.3. Antitussive activity of Codeine phosphate, extracts, soluble fractions against capsaicin




**Capsaicin**

Results in Table 4 revealed that negative control receiving only 3% Tween produced high latent period of cough of 95.15±0.06 and number of cough frequencies of 82.56±0.03 while Codeine phosphate used as standard antitussive drug fournished high latent period of cough of 105.63±0.0.3 for its percentage increase of 11.01±0.03% and low number frequencies of cough of 38.65±0.02 for its inhibition to 48.34±0.03%.

The treatment with aqueous SLAR-1 and 80% methanol SLAR-2 extracts caused significant increase and latent period of cough and decrease of cough frequencies in dose-dependent manner (Table 4). Administered all at the highest oral dose of 200 mg/kg bw, these extracts produced significant increase of latent period of cough by 103.25±0.01and 105.62±0.01 for an increase percentage by 8.51±0.04 and 11.00±0.00%, and remarkable decrease of cough frequencies to 47.02 ±0.04 and 45.22±0.3 for an inhibition of 60.00±0.00 and 63.39±0.02% respectively compared to negative control showing low latent period of cough of 95.15±0.06 and high number of cough frequencies of 82.56±0.03 without inhibitorty effect (0.00±0.00%).

By the same way, the use of soluble fractions SLAR-1.1 to-1.4 for treating showed low latent period of cough varying from 79.82±0.04 to 90.41±0.06 and for its inhibition from 17.16±0.03 to 4.98±0.04% and significant decrease of cough frequencies from 42.04±0.04 to 44.68±0.00 for its inhibition from 45.88±0.00 to 49.08±0.01% compared to negative control presenting high latent period of cough of 95.15±0.06 and cough frequencies of 82.56±0.03. Our results were in good agreement with Billah et al., (2021).

**Table 4: Effects of *S. longipedunculata* extracts and soluble fractions, and codeine phosphate on capsaicin-induced cough.**

| Groups | TTT (mg/kg bw) | LPC (seconds) | % ILPC | CF (Number) | % IFC |
|---|---|---|---|---|---|
| NC | 3% Tween 80 | 95.15±0.06 | 0.00±0.00 | 82.56±0.03 | 0.00±0.00 |
| CP | 10 | 105.63±0.0.3 | 11.01±0.03 | 38.65±0.02 | 48.34±0.03 |
| SLAR-1 | 100 | 98.15±0.04 | 3.15±0.01 | 45.56±0.04 | 44.81±0.04 |
| | 200 | 103.25±0.01 | 8.51±0.04 | 47.02±0.03 | 60.00±0.0 |
| SLAR-2 | 100 | 100.04±0.05 | 5.14±0.02 | 41.25±0.05 | 50.03±0.06 |
| | 200 | 105.62±0.01 | 11.00±0.00 | 45.22±0.03 | 63.39±0.02 |
| SLAR-1.1 | 100 | 86.33±0.04 | 9.27±0.03 | 48.05±0.05 2 | 41.80±0.04 |
| | 200 | 89.65±0.02 | 11.03±0.04 | 44.68±0.00 | 45.88±0.00 |
| SLAR-1.2 | 100 | 82.56±0.06 | 13.23±0.05 | 42.31±0.01 | 46.33±0.06 |
| | 200 | 85.82±0.04 | 17.16±0.03 | 45.04±0.04 | 49.08±0.01 |
| SLAR-1.3 | 100 | 92.54±0.03 | 2.74±0.01 | 44.14±0.03 | 44.11±0.06 |
| | 200 | 94.41±0.06 | 4.98±0.04 | 47.50±0.06 | 48.52±0.00 |
| CPSLAR-1.4 | 100 | 84.59±0.06 | 11.10±0.03 | 43.65±03.0 | 44.70±0.03 |
| | 200 | 81.26±0.04 | 14.64±0.00 | 45.60±0.05 | 47.90±0.06 |

See Table 1.

Figure 6 represented the variation of latent period of cough engendered by Codeine phosphate (CP), aqueous SLAR-1 and 80% methanol SLAR-2 extracts and soluble fractions SLAR-1.1 to 1.4 in capsaicin induced cough compared to negative control (NC).

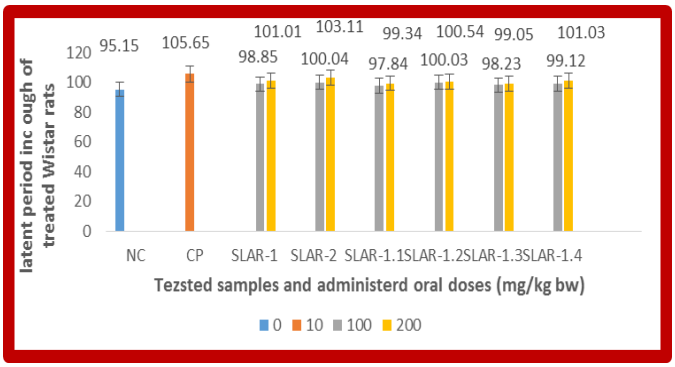



**Figure 6: Variation of latent period of cough produced by negative control (CN), Codeine phosphate (P), aqueous SLAR- and 80% methanol SLAR-2, and soluble fractions SLAR-1.1 to 1.4 observed in capsaicin induced cough.**

It was observed that aqueous SLAR-1 and 80% methanol SLAR-2 extract reacted as Codeine phosphate by the stimulation of the increase of latent period of cough while all soluble fractions increase also this parameter with or not significant difference ($p < 0.05$ or $p > 0.05$) according the case (Fig. 6). Their effects on the latent period of cough was high compared to negative control (Fig. 6).

Figure 7 showed the change of the number of cough frequencies induced by the oral administration of Codeine phosphate (CP), aqueous SLAR-1 and 80% methanol SLAR-2 extracts and soluble fractions SLAR-1.1 to 1.4 in capsaicin-induced coughing.

Aqueous SLAR-1 and 80% methanol SLAR-2, and soluble fractions SLA-1.1 to1.4 delayed significantly the production of cough by the reduction considerably the production of the number of cough frequencies compared to negative control ($p < 0.05$) and exerted antitussive activity. 80% methanol SLAR-2 extract exhibited high activity (CF = 47.04 and 49.87 at 100 and 200 mg/kg bw respectively) comparatively to aqueous SLAR-1 extract (CF = 45.56 and 47.76 at the same oral doses respectively) while soluble fraction ethylacetate SLAR-1.2 showed high activity since it produced low NCF at all tested oral administered doses 100 and 200 mg/kg bw of 42.56 and 44.61 respectively compared to other remaining soluble fractions SLAR-1.1 to 1.4 showing CP varying from 39.66 to 42.56 and 42.47 to 44.83 administered at 100 and 200 m/kg bw respectively compared to negative control producing 82.56 NCF (Fig. 7).

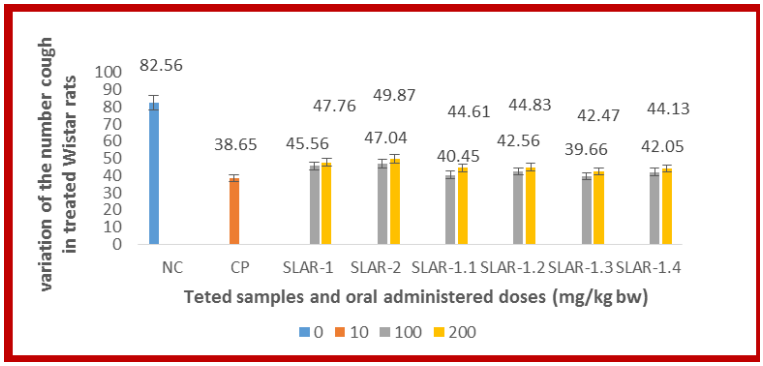



**Figure 7: Change of cough frequencies produced by negative control (CN), Codeine phosphate (CP), aqueous SLAR-1 and 80% methanol SLAR-2 extracts, and soluble fractions -1.1 to 1.4 observed capsaicin induced cough.**

The reported values for latent period of cough and the number of cough frequencies in the present study were in same ranges compared that those reported by Billah et al., (2021). In all antitussive tests, 80% methanol SLAR-2 extract exhited high antitussive activity compared to aqueous extract SLAR-1, which in turn showed high activity compared to soluble fractions suggesting that these last samples can react in synergistic manner to restore the activity of the parent extract. It was observed that after treatment of rats presenting low cough frequencies at the highest oral dose of 200 mg/kg bw, the use of three consecutive days, twice per dag of the same highest oral dose completely stopped the cough

production without recidivity after one week of observation.

In all antitussive tests performed in the present study, it was also observed that Codeine phosphate, aqueous SLAR-1 and 80% methanol SLAR-2 extracts, soluble fractions SLAR-1.1 to -1.4 as well as crude polysaccharide CPSLAR were able to reduced significantly the number of cough frequencies and increase latent period of cough at different extents and these effects clearly demonstrated that all tested samples possessed thus, interesting antitussive property. The increased latent period of cough and the cough suppressive effects as results of aqueous SLAR-1 and methanol 80% SLAR-2 extracts, soluble fractions SLAR-1.1 to 1.4 and crude polysaccharides at the experimental used treatment doses, were weak in all evaluated antitussive tests compared to standard Codeine phosphate antitussive drug (Tables 1 to 3, Fig.1 to 7). Our results were in good accordance with Guo et al., (2016) and Petros, 2020).

The mechanism by which extracts, soluble fractions and crude polysaccharides from *S. longepedunculata* roots influenced experimental cough reflex was not fully understood in the present study. But, it was assumed that observed cough suppressive effect of these samples may be associated with their ability to increase production of mucus in the airways, whether direct or indirect effects via vago-vagal reflex and suppress experimentally induced cough reflex through this co-called barrier mechanism as also previously reported by Fleskova et al., (2012) for the antitussive mechanism of polysaccharides isolated from *Athatoda vasica* leaves. Increased production of mucus may hamper the access of irritating stimulus to airway mucosa.

Research on various medicinal plants properties and identification of active constituents responsible for their divers biological activities had supported the traditional uses of ancient healing knowledge and had proven the continuing healing potential of many medicinal plants. The correct identification of the crude herbal material and standard extracts, the validation of the popular and commun uses and their safety, were some elements that contributed to the development and rational use of different phytomedicines in the world (Reynoso et a., 2017: Petros, 2020).

The antitussive animal model induced particularly by sulfur dioxide and citric acid aerosol in guinea pig had obvious cough reflex, easily observed and required trained observers to monitor carefully the treated animals because their cough reflex was easily observed in the present study contrary, but the central organization of cough was poorly understood as previously reported by Widdicombe (1995).

In the present study, the obtained results suggested that *S. longepedunculata* extracts, soluble fractions and crude polysaccharides might also potent phlegm eliminating effects. However, the administered both doses of tested samples in all antitussive tests performed in the present study, showed their potent antitussive effects since they reduced significantly the number of cough frequencies induced in treated rats as already mentioned above.

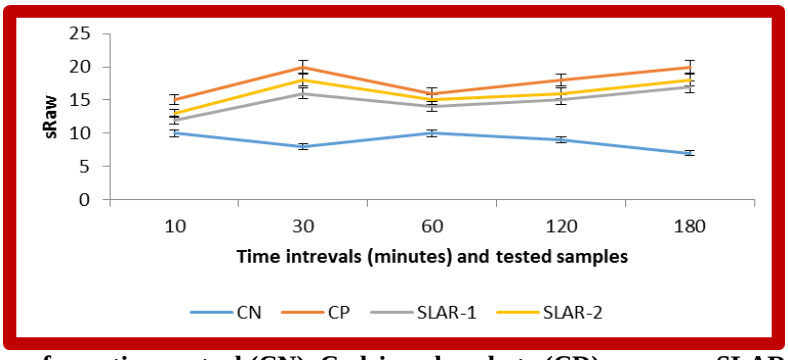



**Figure 8: The influence of negative control (CN), Codeine phosphate (CD), aqueous SLAR-1 and 80% methanol SLAR-2 extracts on citric acid ($C_6H_8O_7$)-induced changes of specific airway resistance (sRaw) *in vivo* conditions, registered before any agent application (values labelled as N in graphs) and after that in 30, 60, 120 and 180 minutes time intervals.**

Airway resistance was a concept used in respiratory physiology to describe mechanical factors which limited the access of inspired air to the pulmonary alveoli. It was a concept used in respiratory physiology to describe mechanical factors which limited the access of inspired air to the pulmonary alveoli. It is generally accepted that bronchodilating compounds can cause cough suppression. Therefore, the evaluation of the changes of specific airway resistance was considered as an indicator of this activity. Our results suggested that the application of extracts, soluble fractions and crude polysaccharides of *S. longepedunculata* roots in the doses which provoked cough suppressive activities did not significantly change the values of specific airway resistance (Fleskova et al., (2012) (Fig. 8).

Our results suggested that the application of extracts, soluble fractions and crude polysaccharides of *S. longepedunculata* roots in the doses which provoked cough suppressive activities did not significantly change

the values of specific airway resistance (Fig. 8). Our results were only qualitatively in good agreement with Fleskova et al., (2012) and Nosalova et al., (2013).

Similarly**,** Codeine phosphate, aqueous SLAR-1 and 80% methanol SLar-2 extracts decrease the number of cough efforts and suppressive effect of cough production was observed after 30 minutes of samples application and persisted until to the end of the experiment up to 180 minutes from their applications.

Moreover, extracts and soluble fractions from various medicinal plants had been the subject of many studies for very long time, especially because of their physical properties, chemical and physical modifications and applications. They could represent an effective alternative treatment to synthetic drugs, because they decrease the parameters of mechanically and chemically induced cough reflex comparatively or even more in comparison with efficacy of peripheral antitussive drugs (Fleskova et al., 2012).

Reported results from our experiments also confirmed these facts and these findings clearly indicated that extracts, soluble fractions and crude polysaccharides from *S. longepedunculata in vivo* conditions, had significant antitussive effect comparable to that of oldest and most active cough suppressive drug Codeine phosphate.

The mechanism by which plant extracts and soluble fractions from *S. longepedunculata* influenced experimentally induced cough reflex was not yet fully understood. But, it was assumed that observed cough suppressive effect of extracts, soluble fractions and crude polysaccharides of *S. longepedunculata* may be associated with their ability to increase production of mucus in the airways, whether direct or indirect effects via vago-vagal reflex as also reported by for polysaccharides isolated for *Adhatoda vasica* leaves. In addition, mechanism of cough suppressive effect of these samples was probably identic with the action of the herbal mucilages, while it was known that they were able to create a protective layer of mucous membranes, especially in viral diseases of upper respiratory tract lading to reduced stimulation of cough receptors (Fledskova et al., 2012).

Also, it was known that many plant extracts and soluble fractions including those observed in the present study, suppressed experimentally induced cough reflex through their so-called barrier mechanism. Hypersalivation subsequently, led to activation of swallowing which together with the cough reflex, shared some central coordination mechanisms (Fleskova et al., 2012). Similarly, these facts can be applied in observed suppression of cough after treatment of studied extracts, soluble fractions and crude polysaccharides from *S. longepedunculata* roots in the present study.

On the other hand, inhalation of capsaicin nebulized spray caused central respiratory depression and fatal apneas mediated via TRPV1 (Transient Receptor Potential Vanilloid-1) activation on lung afferents, pinal cord-ascending tracts and medullary structures including nucleus tractus solitarus. AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor), receptor-triggered conductances acted a vital role n capsaicin-induced apneas (Ren et al., 2017; Billah et al., 2021). Thus, the drug development can be targeted at reducing AMPA receptor-mediated glutamatergic signaling. The opoid Codeine phosphate had been in the core of the cough treatment for long and thus, regarded as the s standard cough suppressant. Codeine phosphate restricted the tracheal constriction along with cough reflex in addition to its central cough suppressant action (Bosler et al., 2007; Billah et al., 2021).

In these experiments, a strict selection procedure was used for the rats enrolled in the study to ensure the exclusion of insensitive or oversensitive treated rats having low or high cough threshold were not entertained for further studies as also previously reported by Petros, (2020) for *Adhatoda schiperiana* (Hoscht,) Nees on ammonia-induced coughing in animals.

In all antitussive tests, data were represented as mean± SEM, standard error mean (SEM) against administered oral doses (mg/kg bw). The comparison of all groups against negative control was performed alongside one way analysis of variation (ANOVA) represented by value for individual group and combination groups respectively denoted $p \leq 0.05$ considered as satistically significant using Student's t test.

In general, in previous studies on antitussive activity (Chimala and Deba, 2004; Guta et al., 2009, 2014; Ashutosh et a, 2012; Fleskova et al. 2012; Hicnjal et al., 2013; Dicpinigaitis et a., 2014; Gupta et al., 2014; Billah et a., 2021), it was observed that medicinal plants producing high or low levels of latent period and low cough frequencies evaluated together in all antitussive tests, were considered to possess antitussive propriety based mainly in the interpretation of the levels of their cough frequencies without keeping account the level of latent period of cough. This meaned or implied that the levels of cough frequencies was an important element to take in account to decide this activity showed by medicinal plant extracts inside latent period of cough that will played an important role that was not clearly specified until now in the published papers and seemed also to be considered with some importance in the manifestation of antitussive activity of tested samples. Sometimes, the number of cough frequency levels were only evaluated and the conclusion was easily taken.

**3.4. Expectorant activity:** Expectorant activity was evaluated by measuring the amount of phenol red secretion and results were presented in Table 5. Salbutamol as reference product produced 8.20 µg/ml of

tracheal phenol red provoking a high percentage increase of tracheal phenol red (TPR) of 104.48±0.02%. At all administered oral doses, extracts, soluble fractions and crude polysaccharides provoked significant increase of tracheal phenol red in dose-dependent manner (Table 5).

Indeed, at the highest oral dose of 200 mg/kg bw, aqueous SLAR-1 and 80% methanol SLAR-2 extracts enhanced the tracheal phenol red output to 8.15±0.05 and 8.56±0.04 µg/mg for a high enhancement percentage of 103.04±0.04 and 113.42±0.00% respectively, compared to negative control showing 4.01±0.03 µg/ml with significant difference ($p < 0.05$).

Also, treatment of rats with soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous SLA-1.1 to -1.4 at the highest oral dose of 200 mg/kg bw, also significantly increased tracheal phenol red from 6.81±0.03 to 7.78±0.0 µg/ml respectively and showed percentage increases in this production from 69.82±0.02 to 94.01±0.04% respectively, with significant difference ($p < 0.05$). 80% methanol SLAR-2 extract showed high expectorant activity compared to aqueous SLAR-1 extract and while soluble fraction ethylacetate SLAR-1.2 exhibited high expectorant activity compared to the remaining soluble fractions SLAR-1.1, SLAR-1.3 and SLAR-1.4 (Table 5). Crude polysaccharides produced 7.12±0.04 TPR for an increase percentage of 77.55±0.05% and its expectorant effect was low compared to aqueous SLAR-1, 80% methanol SLAR-2, soluble fractions ethylacetate SLAR-1.2 and residual NC: negative control, OD: oral dose, OD, optique density, TPR: tracheal phenol red aqueous SLAR-1.4 and was high compared to remaining soluble fractions SLAR-1.1 and 1.3 (Table 5). Crude polysaccharide CPLSAR showed weak expectorant effect compared to aqueous SLAR-1 and 80% methanol extracts, and soluble fractions ethylacetate SLAR-1.2 and aqueous SLAR-1.4 and was high compared to remaining soluble fractions SLAR-1.1 and 1.3 (Table 5).

**Table 5: Effects of *S. longepedunculata* extracts and soluble fractions on tracheal phenol red output in rats (Expectorant activity).**

| Groups | TTT OD (mg/kg bw) | OD (546 nm) | Levels of tracheal phenol red (µg/ml) | Increasing TPR (%) |
|---|---|---|---|---|
| NC | | 0.079±0.05 | 4.01±0.03 | 0.00±0.00 |
| Salbutamol | 4 | 0.166±0.04 | 8.20±0.04 | 104.48±0.02 |
| SLAR-1 | 100 | 0.082±0.06 | 7.25±0.01 | 80.80±0.00 |
| | 200 | 0.079±0.03 | 8.15±0.05 | 103.24±0.04 |
| SLAR-2 | 100 | 0.080±0.04 | 7.62±0.02 | 90.02±0.02 |
| | 200 | 0.077±0.03 | 8.56±0.04 | 113.46±0.00 |
| SLAR-1.1 | 100 | 0.091±0.05 | 6.56±0.00 | 63.60±0.05 |
| | 200 | 0.089±0.03 | 7.00±0.04 | 74.56±0.03 |
| SLAR-1.2 | 100 | 0.084±0.06 | 7.02±0.05 | 75.06±0.00 |
| | 200 | 0.080±0.03 | 7.78±0.03 | 94.01±0.04 |
| SLAR-1.3 | 100 | 0.092±0.05 | 6.25±0.01 | 55.86±0.06 |
| | 200 | 0.090±0.04 | 6.81±0.06 | 69.82±0.02 |
| SLAR-1.4 | 100 | 0.086±0.04 | 6.85±0.04 | 70.82±0.05 |
| | 200 | 0.084±0.02 | 7.85±0.03 | 95.76±0.03 |
| CPSLAR | 100 | 0.080±0.00 | 6.67±0.03 | 66.33±0.04 |
| | 200 | 0.078±0.03 | 7.12±0.04 | 77.55±0.03 |

The expectorant activity exhibited by all tested samples seemed to be robust. The expectorant effect of both extracts, soluble fractions and crude polysaccharides from *S. longepedunculata* roots at the highest oral dose of 200 mg/kg bw, was better, but weak compared to Salbutamol used as reference product (Table 5).

Expectorant drugs were found to increase the secretion of mucins and/or mucus hydratation to an extent which produced a sufficient mass of mucus to be coughed up or sneezed to expel the mucus from the lungs or upper respiratory tracts (Balsamo et al., 2010; Zanad et al., 2017; Billah et al., 2021).
These results suggested that expectorant effects showed by tested extracts, soluble fractions and crude polysaccharides from *S. longepedunculata* roots may be related to their ability to increase tracheobronchial mucus secretion and decrease the viscosity of mucus. Our observation was in good agreement with Guo et al., (2016) who had previously reported the same effect on expectorant activity of 95% ethanol extract of *Potentlla anserine* roots. Also, it was well known that most expectorant drugs increased the secretion and diluted the sputamentum in the respiratory tract such that it was easily expectorated with ciliary movement (Han et al., 2010; Guo et al., 2016) as it was also suspected the same effect for studied samples from *S. longepedunculata* roots. These results suggested that *s. longepedunculata* extracts and soluble fractions, might also potent phlegm eliminating effects as already mentioned above.

Extracts and soluble fractions from *S. longepedunculata* roots caused significantly decrease of tracheal phenol red compared to Salbutamol, excepted extract SLAR-2 showing high activity compared to this compound, but their effect was high compared to negative control showing only 4.10 tracheal phenol red and significant difference was observed ($p < 0.05$). Aditionnally, the increase in the level of tracheal phenol red liked to an increase hexose level in the lung fluid.

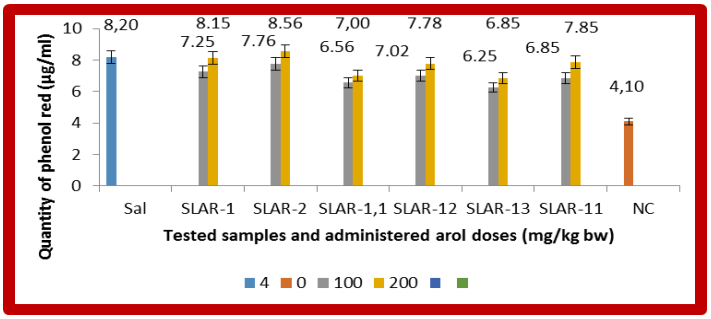



**Figure 9: Secretion of tracheal phenol red observed in rats trachea against treatment of aqueous SLAR-1 and methanol SLAR-2 extracts, and soluble fractions SLAR-1.1 to 1.4 form *S. longepedunculata* roots.**

This indicated a triggered mucin secretion from mucus cells and submucosal glands and goblet cells on the surface epithelium in airways. The intratracheal secretion of phenol red was also significantly increased by both parasymthomimetics and sympathomimetics as it was well known. In the present study, it was observed that all administered samples significantly enchanced tracheal phenol red in dose-dependent manner with the better effect observed with the administration of the highest oral dose of 200 mg/kg bw, and exerted thus expectorant activity as also previously reported by Billah et al., (2021) for the formulated leaf-roots extract mixtures of *Ocimum santum* in their conjugaison with standards and our results were in good agreement with these cited authors. Our results were only qualitatively in good agreement with Song et al., (2015); Kuang et al., (2018) and Billah et al., (2021).

In all antitussive tests**,** it was observed that all tested saM//mples from *S. longependuncuta* roots including Codeine phosphate as standard antitussive reference drug, showed significant increase of the latent period of cough and reduction of the number of cough frequencies and increase of tracheal phenol red compared compared to negative control with significant difference ($p < 0.05$), expressing thus their good antitussive and expectorant activities in the present study.

The maximum antitussive and expectorant activities of tested samples was observed at the highest administered oral dose of 200 mg/kg body weight. Taking account of the antitussive levels showed by soluble fractions and crude polysaccharides, it can be speculated or suggested that responsible active principles of *S. longepedunculata* may be flavonoids and polysaccharides since these phytochemical groups exhibited high and interesting antitussive activity in all evaluated antitussive tests in the present study.
This finding on *Securdata longepedunculata* roots extracts might be helpful for reducing cough supporting and justifying the claimed traditional use of the studied plant part for the symptoms respiratory illenesses.

### 3.5. Acute and subacute toxicity of aqueous extract of *S. longepedunculata*

**3.5.1. Acute toxicity:** The acute toxicity investigation of the aqueous extract of *S. longepedunculata* revealed that no sign of toxic effects such as alteration of the locomotion activity, change in behaviour, and physiological activities, gastrointestinal disturbances appearance, sensory nervous system responses or other abnormalities in treated animals at the administered highest oral dose of 5000 mg/kg bw after 28 days of observation. No change in intake food and water consumption was also observed. The determination of these parameters seemed to be important to the study the safety and tolerability of a therapeutic substance, as proper intake of nutrients and water which were essential to the physiological status of the treated animals and to the accomplishment of proper response to the tested products (Feres et al. (2006).

The extract did not induce any death of treated animal after 28 days of observation at the highest oral dose of 5000 mg/kg bw. Our results were in good agreement with Haruna et al., (2013) who had previously reported that aqueous extract from *S. longpenduculata* root administered at the highest oral dose of 3000 mg/kg, did not induce the mortality of treated animal and its LD50 was estimated to be greater than 3000 mg/kg bw. Therefore, the $LD_{50}$ of the extract was estimated to be greater than 5000 mg/kg bw. Thus, according to the study of Kennedy et al., (1986), substances that presented $LD_{50}$ higher than 5000 mg/kg bw via oral route, may be considered as practically non-toxic and regarded as being safe. This finding suggested that aqueous extract of *S. longepedunculata* was practically non-toxic by oral route and was considered as totally safe and can be used for a long time with large security. Other authors suggested that chemical substance with $LD_{50}$ within the range 5000-15.000 mg/kg bw was also

considered as practically non-toxic (Loomis and Hayes, 1996) and our results remained well in this interval.

**2.3. Sub-acute toxicity of the aqueous extract of *S. longepedunculata*:** In this test, it was observed that the animals feeding the aqueous extract of *S. longepedunculata* at all administered oral doses were healthy. No unusual changes in behaviour, and locomotion activity as well as no ataxis and no other signs of intoxication and abdormalities were observed during 28 days period of observation as reported above indicating the safety, tolerability and innocuousness of the administered aqueous SLAR-1 extract from *S. longepedunculata* roots in treated Wistar rats. There was no significant difference in intake food and consumption of water between treated and untreated animals, but animals which have received extracts at all tested oral doses gained body weight compared to the negative control group (Fig.10) for the same reasons evoked above and significant difference was observed ($p < 0.05$). No death of animal was recorded at all daily administered oral doses suggesting also the total safety, tolerability and innocuousness of administered aqueous SLAR-1 extract in treated rats as already mentioned above. According to Pieme et al. (2006), the progressive increase in body weights during the period of treatment may indicate the improvement of the nutritional state of treated animals and in some cases, it might be attributed to the appetite stimulation of the administered aqueous SLAR-1 form *S. longepedunculata* extract as previously reported by Ogbonnia et al., (2009). The growth response effect could be considered as a result of the increased food intake and water consumption.

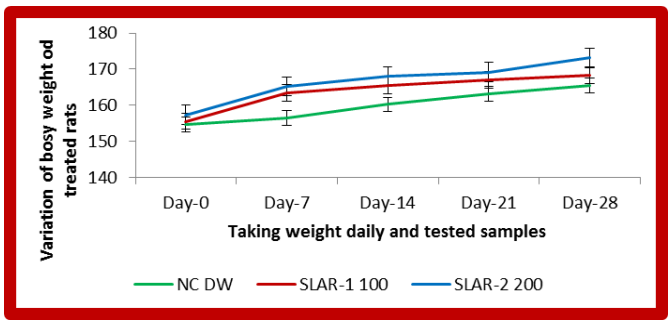



**Figure 10: Variation of body weights of treated rats in the presence of aqueous SLAR-1 and 80% methanol SLAR-2 extracts.**

**2.3. Effects of the aqueous extract of *S. longepedunculata* on some haematological parameters:** The haematological parameter profile of treated Wistar rats ath the highest oral dose of 5000 mg/kg bw was presented in Table 2. The data of this investigation revealed that the haemoglobin and red blood cells (RBC) levels of treated rat groups were significantly increased with significant difference ($p < 0.05$) compared to untreated group. This may be due to the increased absorption of iron and copper or to the immunopotentiating effect of the extract as also previously reported for some medicinal plant extracts (Ameyaw and Owusu-Ansah, 1998) (Table 2). Thus, the extract induced erythropoiesis and the iron was incorporated into the haemoglobin. Further, the iron containing supplementations can be used to increase MCV (mean corpuscular Value) values of RBC in anemic conditions caused to poorer intake of iron containing food (Scholl et al., 1992).

The level of haematocrit did increased in treated Wistra rats and show significant change ($p < 0.05$) compared to negative control group and. It was observed significant increase of WBC (white blood cells) animals compared to untreated group ($p < 0.05$) (Table 6).

The haematopoietic system is one the most sensitive targets for cytotoxic substances and an important index of physiological and pathological status in humains and animals (Mukinda and Syce, 2007). In the present study, a significant decrease of platelet level was observed in treated animals compared to untreated.

**Table 6: Effects of the aqueous extract of *S. longepedunculata* on the levels of some haematological parameters at oral dose of 5000 mg/kg body weight.**

| Parameters | Negative control | *S. longepedunculata* 5000 mg/kg bw | Reference values |
|---|---|---|---|
| RBC (x $10^6$ $\mu L^{-1}$) | 8.2 ± 0.5 | 9.5 ± 0.2 | 7.6-10.29 |
| Hemoglobin (g/dL) | 16.2 ± 0.0 | 17.8 ± 0.0 | 15-18.2 |
| Hematocrit (%) | 46.6 ± 0.3 | 47.9 ± 0.6 | 40.7-50 |
| Platelets (x $10^3$ $\mu L^{-1}$) | 1278.0 ± 0.6 | 1228.5 ± 0.4 | 995-1713 |
| WBC (x $10^3$ $\mu L^{-1}$) | 14.7 ± 0.4 | 18.2 ± 0.5 | 6.6-20.5 |
| Neutrophils (%) | 21.5 ± 0.3 | 22.8 ± 0.3 | 3-24.7 |
| Basophils (%) | 0.0 | 0.0 | 0.0 |
| Eosinophils (%) | 0.8 ± 0.1 | 1.2 ± 0.6 | 0-2 |

| | | | |
|---|---|---|---|
| Lympocytes (%) | 85.2 ± 0.6 | 90.7 ± 0.4 | 58.8-94 |
| Monocytes (%) | 3.2 ± 0.1 | 3.6 ± 0.2 | 0-4 |
| Segmented leucocytes s (%) | 17.6 ± 0.6 | 22.6 ± 0.2 | - |

RBC; red blood cells, WBC: white blood cells

This indicated that *S. longepedunculata* aqueous SLAR-1 extract had an small effect on the production of platelets or induced thrombocytopenia (reduction in the number of platelets in the blood). Additionally, with a decrease of this parameter count, there is an increased risk of bleeding (Slarichter, 2004).

Taken together, this suggested that aqueous extract of *S. longepedunculata* had an anti-haematopoiesic activity. This observation of decreased platelets level in circulatory system by the extract also means that it had anticoagulant property as also reported for other medicinal plant extracts (Adedapo et al., 2008; Li et al., 2010; Mukinda and Eagles, 2010; Ajugwo and Ezimah, 2013; Gandhare et al., 2013; Kikweta et al., 2015).

The remaining evaluated haematological parameters such as neutrophils, eosinophils, lymphocytes and segmented leucocytes showed significant increase in treated animals according to the case, with significant difference ($p < 0.05$) compared to negative control group.

**2.4. Effects of the aqueous extract of *S. longepedunculata* on some biochemical parameters:**

Table 7 showed the effects of the oral administration of the aqueous extract SLAR-1 from *S. longepedunculata* at the highest oral dose of 5000 mg/kg bw on the levels of some biochemical parameters of treated Wistar rats.

Results indicated that the oral administration of the extract at the highest oral dose of 5000 mg/kg bw in acute toxicity induced significant decrease of the level of glucose in treated groups compared to that seen in untreated group ($p < 0.05$). This decrease may be due probably to the hypoglycemic properties of the extract as also previously reported for other medicinal plant extracts (Ogbonnia et al, 2008; Kripa et al., 2011. Nsaka et al., 2013; Cimanga et al., 2016) an may be exploited for the treatment of diabete type 2 in traditional medicine (Cimanga et al., 2016).

ALAT and ASAT are two liver enzymes associated in the hepatocellular damages and were thus considered as indicators of liver damages. They were also known as enzymes used as good indicators of liver function (Hilaly et al., 2004) and as biomarkers predicting possible toxicity of this vital organ (Mukinda and Eagles, 2010; Cimanga et al., 2015; Kikwueta et al., 2015).

**Table 7**: **Effects of the aqueous extract of *S. longepedunculata* on the levels of some biochemical parameters at oral dose 5000 mg/kg body weight.**

| **Parameters** | **Negative Control** | ***S. longepedunculata*: 5000 mg/kg bw** |
|---|---|---|
| Glucose (mg/dL) | 242.5 ± 0.4 | 235.3 ± 0.4 |
| Creatinine (mg/dL) | 0.87 ± 0.05 | 0.86 ± 0.02 |
| AST (UI/L) | 177.6 ± 0.3 | 178.2 ± 0.5 |
| ALT (UI/L) | 50.2 ± 0.2 | 49.5 ± 0.2 |
| Triglycerides (mg/dL) | 43.7 ± 0.8 | 43.3 ± 0.5 |
| Total bilirubin (mg/dL) | 0.5 ± 0.1 | 0.4 ± 0.7 |
| Direct bilirubin (mg/dL) | 0.2 ± 0.0 | 0.2 ± 0.0 |
| Total Proteins (g/dL) | 7.6 ± 0.3 | 8.1 ± 0.1 |
| Albumin (g/dL) | 3.4 ± 0.5 | 3.5 ± 0.6 |
| ALP (IU/L) | 145.4 ± 0.6 | 146.3 ± 0.4 |
| Total cholesterol (mg/dL) | 101.2 ± 0.3 | 98.6 ± 1.2 |
| HDL-cholesterol (mg/dL) | 62.3 ± 0.3 | 65.6 ± 0.3 |
| LDL-cholesterol (mg/dL) | 37.5 ± 0.1 | 35.2 ± 0.4 |
| Uric acid (mg/dL) | 1.91 ± 0.1 | 2.1 ± 0.5 |
| Urea (mmol/L) | 5.1 ± 0.8 | 6.9 ± 0.6 |

AST: aspartate transferase, ALT: alanine transferase, ALP: alkaline phosphate, HDL: hight-density lipoproteins, LDL: low-density lipoproteins.

Although both ASAT and ALAT were common liver enzymes, only ALAT was remarkably specific for liver function and ASAT was mostly present in wide variety of tissues including the heart, the myocardium, skeletal muscle, kidneys, liver and brain (Wasan et al., 2001; Crook et al., 2006; Kripa et al. 2011; Kiwueta et al., 2015). The analysis of these parameters was important since there were several reports of liver and kidneys toxicity related to the use of phytotherapeutic products needing the prevention of the occurring of this evenement (Ozolua et al., 2009; Kripa et al., 2011). These transaminases were well-known as enzymes used as good indicators of liver and kidneys function and biomarkers predicting possible toxicity of these both

vital organs. Also, the elevation of levels of the both transaminases in the blood resulted in any damage to parenchymal liver cells (Mukinda and Eagle, 2010).

Results reported here indicated that there was slight increase of the levels of these both enzymes, but, it did not show significant difference ($p > 0.05$) compared to the negative control. This finding implied that the aqueous SLAR-1 extract administered at the highest tested oral dose of 5000 mg/kg bw, may not cause damages of these organs cited above (Pieme et al., 2006; Lima et al., 2009; Ogbonnia et al., 2009),. Based on this, it was also suggested that hepatocytes of the treated rats were not damaged, the hepatic and renal functions of the treated animals were maintained safe as this effect can be also considered for other organs since the extract did not possess significant deleterious effects in treated animals on these organs as also previously reported for other medicinal plant extracts (Akharainyi et al., 2012; Nsaka et al., 2013; Cimanga et al., 2015).

The level of creatinine as hepatic biomarker enzyme of treated groups did not show significant difference compared to untreated groups ($p > 0.05$) and suggested that aqueous extract SLAR-1 of *S. longepedunculata* roots did not possess significant deleterious effects on hepato-renal functions or again and the function of liver and kidney of treated mice was not altered (Kripa et al., 2011; Akarainyi et al, 2012, Coolborn et al., 2012; Rajasekaran and Kannabiran, 2012). There were no adverse effects on the usual markers of liver and kidney toxicity, it may be concluded that the administered aqueous extract SLAR-1 did not induce significant damage to these organs showing its safety and tolerability on these two vital organs as already mentioned above.

The observed slight decrease of the level of cholesterol, LDL and triglycerides with significant difference ($p < 0.05$) in treated rat groups compared to untreated rat groups, may be due to the hypolipidimic properties of the extract and in sometimes, to the increase of the secretion of thyroid hormones T3 and T4 (Arüjo et al., 2005). Significant increase in HDL levels ($p < 0.05$) in treated animals compared to untreated animals, was observed, but it does not show significant difference ($p > 0.05$). The increase HDL, decreased of total cholesterol and LDL were of greater significance in cardiovascular diseases management (Akpanabiatu et al., 2013). Thus, these results suggested that the extract had some beneficial effects by reducing cardiovascular risk factors which contributed to the death of mainly diabetic patients (Ameyaw and Owusu-Ansah, 1998; Ogbonnia et al., 2008; Akpanabiattu et al., 2013; Cimanga et a., 2015; Kikweta et al., 2015).

Albumin was a protein with high level in plasma. Since it was produced in the liver, its decrease in serum may arise from liver and kidney diseases (Lima et al., 2009). Fortunately, this was not observed in the treated rats in the present study, its level in treated animals was comparable to that of untreated rat groups and did not show statistically significant difference ($p > 0.05$). In addition, there was not significant change observed in the levels of the total and direct bilirubin in treated animals compared to control group ($p > 0.05$) suggesting that jaundice could not be occurred in the intake of this aqueous SLAR-1 extract from *S. longipendunculata* as also reported by Coolborn et al. (2012) for ethanol extract of *Spathodea campanulata* leaf. The total proteins level significantly increased in treated rats compared to untreated group ($p < 0.05$) suggesting an apport of an exterior supply of this element, mainly from the administered aqueous SLLAR-1 extract from *S. longepedunculata* roots.

The urea level significantly increased at all used oral doses in treated groups compared to untreated groups ($p < 0.01$), but this last observation was not found as a sign of insufficiency renal because their level remained within the normal limits (2.5– 7.5 mmol/L). As urea production in mammals occurred specially in liver, its level could also be used as an indicator of hepatic function and more confirmed the good state of this vital organ (Arüjo et al., 2005). Thus, our results more suggested and confirmed good hepatic function of treated animals as already demonstrated above with the levels of other hepatic biomarkers. Together, the normal values of renal biochemical parameters including urea, uric acid and creatinine suggested that the aqueous SLAR-1 *S. longepedunculata* extract did not produce any sort of disturbances in the liver and kidney functions, as also been found in the case of other various medicinal plant extracts (Saha et al., 2011; Rajasekaran and Kannabiran, 2012; Akpanabiatu et al, 2013; Gandliare et al., 2013).

Serum ALP was a sensitive detector for intrahepatic and extrahepatic bile obstruction. From the obtained results, no significant difference in the level of ALP in treated rat groups compared to untreated group was recorded although an slight increase was observed in treated groups with no significant difference ($p > 005$) (Table 1). As the presence of infiltrative diseases of the liver and all bones diseases was associated with osteoplastic activity (Vasudevan and Sreekumari (2005), it was likely observed that the oral dose used in this study for the aqueous extract of *S. longepedunculata* roots did not abnormally interfere with the calcification or metabolic activities involving the liver. This finding was in good agreement with Pieme et al. (2006) and Eden and Usoh (2009) concerning the effect of other medicinal plant extracts on ALP level in treated animals.

In general, all levels of haematological and biochemical parameters evaluated in the present study were within the acceptable physiological ranges (Barry et al., 1995: Feldamn et al., 1997; Cimanga et al., 2015).

Table 8 showed the influence of the aqueous extract SLAR-1 from *S. longepedunculata* of on some

electrolytes. Results revealed that the administration of aqueous extract SLAR-1 at all oral doses induced significant increase of calcium, chloride, iron, potassium and sodium levels in treated animals compared to untreated animals ($p < 0.05$) with statistically significant difference between both groups ($p < 0.05$).

A statistically significant difference in decrease of inorganic phosphorus level ($p < 0.05$) was also observed in treated animals compared to untreated animals (Table 8). In addition, the administration of the aqueous extract SLAR-1 at low oral dose of 1000 mg/kg bw did not show significant difference between the weights of all organs of treated animals compared to untreated animals ($p > 0.05$), but this was observed at the highest oral dose of 5000 mg/kg bw with significant difference ($p < 0.05$) (Table 8).

**Table 8**: **Effects of the aqueous extract of *S. longepedunculata* on the levels on some electrolytes (mg/dL) in Wistar rats in acute toxicity.**

| Electrolytes | N. control | *S. longepedunculata* 1000 mg/kg bw | *S. longepedunculata* 5000 mg/kg bw |
|---|---|---|---|
| Calcium | 9.7 ± 0.3 | 10.2 ± 0.4 | 10.8 ±0.5 |
| Chloride | 72.2 ± 0.4 | 77.0 ±0.3 | 79.4 ± 0.4 |
| Inorganic phosphorus | 4.0 ±0.7 | 3.7 ± 0.4 | 4.2 ± 0.3 |
| Iron | 7.4 ±0.2 | 8.8 ± 0.4 | 9.7 ± 0.4 |
| Potassium | 71.5 ± 0.4 | 74.8 ± 0.5 | 77.7 ± 0.6 |
| Sodium | 74.5 ± 0.4 | 77.3 ± 0.7 | 81.2 ± 0.5 |

N. control: negative control

The weights of vital organs were showed in Table 9. Results revealed that the administration of aqueous extract SLAR-1 reached significant increase of these organ weights in dose-dependent manner which was more significant and showed remarkable difference at all administered oral doses of 1000 and 5000 mg/kg bw ($p < 0.05$). This observation suggested that the aquoeus extract SLAR-1 at all administered oral doses had no significant deleterious effects on these treated animal organs and it did not detrimentally affect the weights, organ-to-body weight ratio because the colour and form of organs in subacute toxicity remained totally in good and normal state, maintained in orginal architecture since no deleterious effect was observed in treated Wistar rats compared to untreated rat group.

**Table 9: Effects of the aqueous extract SLAR-1 of *S. longepedunculata* on the organ weights (g/kg) of Wistar rats.**

| Organs | Negative control | *S. longepedunculata* 1000 mg/kg bw | *S. longepedunculata* 5000 mg/kg bw |
|---|---|---|---|
| Brain | 3.70 ± 0.7 | 3.74 ± 0.3 | 3.77 ± 0.4 |
| Heart | 0.92 ± 0.4 | 0.95 ± 0.5 | 0.96± 0.4 |
| Kidneys | 2.31 ± 0.2 | 2.41 ± 0.4 | 2.48 ± 0.2 |
| Lungs | 3.00 ± 0.7 | 3.17 ± 0.4 | 3.21± 0.4 |
| Pancreas | 1.56 ± 0.4 | 1.60 ±0.2 | 1.67 ± 0.7 |
| Spleen | 0.73 ± 0.5 | 0.75 ±0.2 | 0.78± 0.7 |
| Testicules | 9.22 ± 0.4 | 9.44 ± 0.7 | 9.56 ± 0.3 |
| Ovaries | 0.24 ± 0.3 | 0.27 ±0.1 | 0.31 ± 0.4 |

**2.5. Histopathological study:** No significant difference between control group and treated groups in the organ weights of animals was observed. The macroscopic examination of the organs of treated animals with the highest oral dose of 5000 mg/kg bw of the aqueous extract of *S. longepedunculata* did not show any change in form and colour of organs in treated groups compared to negative control group rat. No detectable abnormality such as hypertrophy of organs was also observed in pathological examination of the tissues.

No alterations in cell cultures or unfavourable effects were seen in the microscopic examination of the internal organs using multiple magnification powers. No pathology was recorded in the histological sections of the vital organs such as heart, spleen, kidney, liver and lung. Histopathological examination of the negative control group showed normal architecture and absence of any gross pathological lesions in organs compared to treated groups and more confirmed observations deduced in subacute toxicity on organ weights of treated rats. All treated rats were considered healthy.

## 3. CONCLUSION

The present study reported for the first time the antitussive and expectorant activities of aqueous and 80% methanol extracts, chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions and crude

polysaccharides from *Securidaca longepedunculata* roots in animal model. Results revealed that all tested samples were able to increase latent period of cough, decrease significantly mainly the number of couch frequencies and increase tracheal phenol red of treated Wisatr rats expressing thus their antitussive and expectorant properties. By these effects, these samples showed clearly that they were endowed with antitussive or cough suppressant and expectorant properties with different magnitudes. Aqueous SLAR-1 extract which was the common traditional remedy used in traditional medicine, did not induced mortality of treated rats at the highest oral dose of 5000 mg/kg body weight and their lethal dose was estimated to be greater than 5000 mg/kg body weight. Aqueous extract had not deleterious effects on haematological and biochemical parameters, increased vital organ weights and electroytes of treated rats. These reported results constituted a scientific base supporting and justifying the use of roots of *Securidaca longepedunculata* in decoction form in traditional for the treating of cough and various respiratory disorders in Democratic Republic of Congo and other African countries where the plant part was employed for the same medical purpose.

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