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PHARMACOLOGICAL ASSESSMENT OF ANTISPASMODIC, ANTIOXIDANT AND FREE RADIADL SCAVENGING ACTIVITIES OF EXTRACTS, SOLUBLE FRACTIONS AND ISOLATED CONSTITUENTS FROM *ALSTONIA CONGENSIS* ENGL. (APOCYNACEAE) ROOT BARK

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ABSTRACT

The present study was initiated for the assessment of antispasmodic on acethylcholine and depolarization solution rich in KCl induced contractions on isolated guinea-pig ileum and antioxidant and free radical scavenging activities activities and a large game of reactive oxygen species (ROS) respectively of extracts, soluble fractions and isolated compounds from *Alstonia congenesis* root bark. **In antispasmodic test**, results revealed that aqueous and 80% methanol extracts produced inhibition of contractions induced by acetylcholine and depolarization solution rich in KCl on isolated guinea-pig ileum more than 80% as good activity while soluble fractions produced the same effect in producing 66.85±0.05 to 78.85±0.04% and 64.52±0.02 to 76.56±0.04% inhibition of contractions induced by the same agonist acethylcholine and depolarization solution rich in KCl on isolated organ respectively as moderate and good activity according the case. Isolated compounds boonein, echitamine, 6,7 secoangustilobine and β-amyrine inhibited contractions induced by acethylcholine on isolated organ with percentages from 70.54±0.02 to 82.65±0.00% as good actvity, and against depolarisation rich in KCl with percentages ranged from 68.75±0.04 to 80.48±0.06% as moderate and good activity according the case. **In antioxidant testing**, results revealed that aqueous and 80% methanol extracts inhibited the activities of all selected reactive oxygen species such**,** 1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonate (ABTS), superoxide anions ($O^{2\bullet-}$), hydroxyl ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2) (ROS) with inhibitory concentration 50 (IC_{50}) < 10 µg/ml as pronounced, excepted aqueous extract bending hydrogen peroxide activity with a IC_{50} value of 11.07±0.04 µg/ml as good activities. Chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions inhibited also ROS activities with IC_{50} values ranging from 7.05±0.04 to 15.32±0.04 µg/ml while total phenolic compounds reacted in the same manner by producing IC_{50} values < 10 µg/ml against all selected ROS as pronounced activity and can be considered as responsible for the observed antioxidant activity. These results showed that all tested samples possessed antispasmodic and antioxidant activities expressed at different magnitudes and can be used in treating of diarrhea and gestion of degenerative diseases such as cardiovascular diseases.

KEYSWORDS: *Alstonia congensis*, Apocynaceae, root bark, extracts, fractions, isolated constituents, polyphenols, flavonoids, antispasmodic and antioxidant activities.

1. INTRODUCTION

Gastro-intestinal spasms or disorders are common global problems in all age groups wordwide. It is categorized among the most frequently encountered illenesses in humans that are often characterized by unexplainable gastro-intestinal dysfunctions via organic abdominalities, through marked by persistent or recurrent excruciating pains in the abdomen (Okoye et al., 2023). It is responsible and accountable for the intake and digestion of foods, absorption of nutrients, as well as the excretion of end products of food digestion (Rajesh et al., 2014; Saini et al., 2014; Tambuli et al., 2022).

Gastro-intestinal diseases (GID) are the most common complaints that normally affect the largest proprortion of children, adolescents and adults with overlapping clinical

manifestations in diagnosis and medical needs. Some drugs endowed with antispasmodic activity are currently and normally applied or used for symptomatic treating of contractions and crampings of smooth muscles in gastro-intestinal diseases as well as in other critical clinical situations (Rauf et al., 2021). They include the symptoms in the mid as well as lower part of the digestive tract and can be specified as any disease occurring within gastro-intestinal tract.

The intestinal functional disorders that are part of these problems correspond to chronic digestive symptoms that point to a dysfunction of the lower part of the digestive tract, particularly the small intestine and the colon, and that cannot be explained by any organic abnormality. They are characterized by persistent or recurrent incomprehensible pains in the abdomen (Markrane et al., 2018)

These symptoms include abdominal pains, bloating, colic, constipation, diarrhoea, flatulance, cramping and are mainly due to spams, etc., (Sani et al., 2014). Unfortunately, no single drug exist or has proven to be effective for the treatment of gastro-intestinal disorders leading nowadays to the search of an effective and safe drug to control the motility disturbances (Kumar et al., 2015). These syndromes are frequently observed worldwide and can affect between 15 and 20 % of the population (Markrane eta al., 2018).

On the other hand, irritable bowell syndromes (IBS) are known as functional gastro-intestinal disorders for which the exact causes are unknown although their symptoms comprise constipation, diarrhoea, crampings and abdominal pains. Its treatment is currently conducted by the use of antispasmodic synthetic drugs and medicinal plants endowed with this medical property than can relax smooth muscle and prevent spasms (Hadley and Gaarder, 2005; Wiya et al., 2017). These drugs possess indesirable side effects such as dry mouth, urinary retention, headache, nausea, vomiting and constipation (Borelli et al., 2009 Wiya et al., 2017).

To avoid these contraints or compulsions, the population turns to traditional medicine using different medicinal plants with proved antispasmodic activity to treat gastro-intestinal disorders and found some reliefs or alleviations after a long treatment (Rauf et al., 2021). These medicinal plants and derivative products are used from generation to generation for their nutritional and therapeutic effects as also reported by Rauf et al., (2021) attributed to the presence of different bioactive constituents belonging to different phytochemical groups such as phenolic compounds (flavonoids, anthocyanins, quinones, phenolic acids, tannins, etc.), reducing sugars, steroids, terpenoids, saponins, alkaloids, polysaccharides, lignans, stilbenes, etc. acting alone or in synergistic manner (Rajagopal et al., 2019; Kozlowska e al., 2022).

Nowadays, several medicinal plants are scientifically studied to prove or to establish their antispasmodic activity on different isolated organs and were reported to be endowed with this biological property (Cimanga et al, 2010, 2024; Nisar et al., 2015; Asifa et al., 2017; Martinez-Pérez et al., 2018; Tambuli et al., 2022; Okoye et al., 2023).

On the other hand, the vast majority of complex life on the earth requires oxygen for its existence, oxygen is a high reactive molecule that damages living organisms by producing reactive oxygen species (Sekendar et al., 2013). Reactive oxygen species (ROS) are high free radicals formed by exogenous chemicals and endogenous metabolic processes in human body. There are able or apte of oxiding macrobio-molecules such as dexoxynucleic acid (DNA), lipids and proteins (Cimanga et al., 2024; Mondo et al., 2024). Damages to DNA can cause mutations and possibly some diseases as cancers and other, if not reversed by DNA repair mechanisms, damages of proteins cause enzyme inhibition, denaturation and degradation of proteins and lipids. Therefore, organisms containing complex network of antioxidant metabolites and enzymes that can work together to prevent oxidative damages to cellular components such as DNA, proteins, lipids, etc. (Vertuand et al., 2004; Sekendar er al., 2013).

ROS also have useful cellular functions like as redox signaling, so the function of antioxidant systems is not only to remove oxidants entirely, but also instead to keep them at an optimum level (Sekendar et al., 2013, Lenneke and Cochemé, 2021). Phytochemicals present in a wide range in foods and medicinal plants, play a pivotal role in preventing and treating chronic diseases induced by oxidative stress and by working as antioxidants.

These compounds exhibit potent antioxidant, anti-inflammatory, anti-aging, anticancer, and protective properties against cardiovascular diseases, diabetes mellitus, obesity, and neurodegenerative conditions (Muscolo et al., 2024). Antioxidants are also being studied as possible treatment for neurodegenerative diseases like Azheimer's disease, Parckinson's disease, arterosclerosis, cardiovascular diseases, age-associated diseases, amyotrophic lateral sclerosis and as a way to prevent noise-induced bearing loss (Sekendar et al., 2013; 3, Lakshmibai and Amirtham, 2018; Forman and Zhang, 2021, Bierger et al., 2023, Rauf et al., 2023). Antioxidants inhibit oxidation and help to prevent non-Communicable diseases, such as aging and inflammatory processes, tumors, kidney and liver diseases, coronary heart disease, cataracts, renal toxicity and neurological diseases (Ayoka et al., 2022). It was found that antioxidants in the diet have the capacity to prevent oxidative anxiety-related disorders. Antioxidants can render protection of the body from certain type of cancers, heart diseases and premature ageing. They protect the body against the free radicals which are

usually formed as part of metabolism (Rajagopal et al., 2019). They also act as important chemical defense against herbivores through their specific physiological actions on insects. In addition, by reacting directly with the oxidation products of fatty acids and phenolic compounds, they can prevent adverse changes from the occurring in both living organisms and foods (Kozlowka et al., 2022).

Large epidemiological studies support the relationship between oxidative stress and global health, high consumption of foods rich in antioxidants is associated with lower disease rates and preventive protection. ROS are not only important in host defence, but also paradoxically limit the inflammation and immune response (Brieger et al., 2023, Rauf et al., 2023). These ROS include superoxide anions, hydroxyl hydrogen peroxide, hypochlorous acid, etc. (Walko et al., 2007; Cimanga et al., 2024).

For *Alstonia congensis*, about 15 alkaloids have been isolated from the root bark, stem bark and leaves of this tree with similar uses as *A. boonei* as a diuretic and hypotensive. A bark decoction of *A. congensis* is used to treat malaria, gonorrhoea, diarrhoea and other intestinal problems, rheumatic pain, and as a galactagogue. The bark is also applied as an antidote for arrow poison and as an anthelmintic. Lightly roasted leaves are smoked in a pipe as a remedy for cough. Latex obtained from the plant is used as an adulterant in rubber (*Hevea brasiliensis*) (https://apps.worldagroforestry.org/treedb/AFTPDFS/Alstonia_congensis.PDF, 2023).

Its wood is used for light construction, light carpentry, canoe making, mouldings, furniture, interior joinery, utensils, crates, crates, matches, pencils, sculptures (e.g. masks), as well as for veneers and plywood (interior). It is locally appreciated for making household utensils, due to its ease of work and stability. A decoction of the bark is used to treat malaria, gonorrhea, diarrhoea, dysentery and other intestinal ailments, rheumatic pains, as a galactogen and the bark is also used as an antidote to arrow poison and as a dewormer. Latex is used to treat leucorrhea, ulcers, scabies, yaws and headaches. The lightly roasted leaves serve as pipe tobacco and are a remedy for coughs. The tree is sometimes planted as an ornamental or shade tree (https://prota.prota4u.org/protav8.asp?fr=1&g=pe&p=Alstonia+congensis+Engl, 2023). This medicinal plant is found in Nigeria until Centrafrica Republic, eastern and south of Democratic Republic of Congo and north of Angola (https://fr.wikipedia.org/wiki/Alstonia_congensis, 2023).

Methanol extract of leaves showed some antimalarial action in tests with *Plasmodium berghei i*n mice, it stopped the beginning of this infection, but proved ineffective when the infection was already established. The bark and leaves have shown action on the heart in animal tests. Various alkaloids have been isolated from the bark and leaves. Among them is an indole alkaloid, Echitamine, which has shown various pharmacological actions (e.g. hypotensive action and relaxing action on smooth muscle) in prototype animal tests. However, this compound has shown only very limited efficacy against *Plasmodium falciparum* (https://doi;org/rota.prota4u.org/protav8.asp?fr=1&g=pe&p=Alstonia+congensis+Engl.,2023)

The present study is initiated to assess for the first time, *in vitro* antispasmodic and antioxidant activity of extracts, fractions and isolated compounds from *Alstonia congensis*

2. MATERIALS AND METHODS

2.1. Plant material

Stem bark of *Alstonia congensis* Engl. (Apocynaceae) were collected in Kinshasa in July 2023. The plant was identified at the Institut National d'Etudes et de Recherches en Agronomie (INERA), Department of Biology, Faculty of Sciences and Technology, University of Kinshasa. A voucher specimen was deposited in the herbarium of this institute and another in the laboratory of pharmacognosy and phytochemistry at the faculty of Pharmaceutical Sciences of the same university. The plant material was dried at room temperature for one week and reduced to powder using an electronic blender and the resulting powder kept in a brown bottle hermetically closed before use to avoid contaminations.

Figure 1: Alstonia congensis (leaves, stem and flowers).

2.2. Preparation of extracts and fractionation

20 g of powdered stem bark were macerated with 200 ml of distilled water and boiled on a hot plate for 15 minutes. After, cooling, the mixture was filtered on sterile cotton and paper filter Whatman N° 1 and the resulting filtrate evaporated *in vacuo* giving a dried extract denoted as aqueous extract AC-1 (14.37 g). After, an amount of 10 g of AC-1 were dissolved in 200 ml distilled water and filtered in the same conditions as described above. The resulting filtrate was exhaustively and successively extracted with solvents of different polarities including chloroform, ethylacetate, and *n*-butanol. All fractions including residual aqueous phase were treated as described above yielding corresponding dried extracts denoted as chloroform (AC-1.1: 1.46 g), ethylacetate (AC-1.2: 2.45 g), *n*-butanol (AC-1.3: 2.25 g) and residual aqueous phase AC-1.4 (2.85g).

An new batch of 20 g powdered stem bark were macerated with 80% methanol for 24 h. After fitration to have a macerate, the marc was exhaustively percolated with the same solvent. The collected solution was filtered in the same conditions and mixed with macerate. This mixture was evaporated in *vacuum* to have a dried extract dented 8% methanol extract AC-2 (16.12 g).

2.3. Anitspamodic spasmolytic testing

Adult male guinea-pigs were anesthetized and sacrificed by cervical displacement followed by exsanguination. The ileum was dissected out (2-3 cm long), plentifully washed with distilled water and suspended in an organ bath (50 ml) containing Tyrode's solution (mM: KCl: 2.2, $MgCl_2$:0.11, $NaH_2PO_4.2H_2O$: 0.42, $CaCl_2$: 1.8, NaCl: 137, $NaHCO_3$:11, glucose: 5.6) or depolarizing solution rich in KCl (DSR-KCl) (mM: NaCl: 2.7, KCl: 100, $NaHCO_3$:15, $CaCl_2$: 1.25, $MgCl_2$:12.5, glucose: 11) in the presence of 95% O_2 and 5% CO_2 (Ali et al.;, 2014. Cimanga et al., 2010, 2024; Jovanovic et al., 2024).

The isolated tissue was allowed to equilibrate for 30 minutes under a resting tension of 0.5 g in Tyrode's solution or in DSR-KCl before exposure to drugs and tested samples. To evaluate antispasmodic activity, the tissue was first exposed to 5.10^{-7} M acetylcholine (ACh) or DSR-KCl to have tree equivalent contractions and the tissue was plentifully washed with Tyrode's solution to eliminate the presence of agonist Ach and DSF-KCl in organ bath. 4 ml of Tyrode's solution were removed in organ batch. After 2 ml of each test sample (40 µg/ml) were separately added in organ bath and left in contact with isolated organ for 15 minutes. And after, 2 ml of each agonist Ach and DSR-KCl were immediately and separately added to stimulate isolated organ according the time of the experiment and left in contact with ileum for 15 minutes.

The effects of extracts and soluble fractions on the responses elicited by both agonist Ach and DSR-KCl were recorded. The responses were recorded via a frontal writing lever on kymograph paper (Scientific and Research Instruments Ltd. England) displayed contractions induced by agonists in the presence of tested samples. The experiment was repeated tree times and mean inhibition percentages of both agonist ACh and DSR-KCl contractions in the presence of each tested sample was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Cag- Cts}}{\text{Cag}} \times 100$$

Where Cag is the amplitude level of contractions induced by agonist or DSR-KCl and Cts is the amplitude level of contractions induced by each tested sample in the presence of respective agonist (amplitude expressed in cm) (Cimanga et al., 2010, 2024; Ali et al., 2014).

2.4. Evaluation of antioxidant activity

2.4.1. Determination of DPPH (1,1-diphenyl-2-picryl-hydrazyl)

The ability of free radical scavenging and antioxidant activities of extracts and fractions from *T. gilletii* leaves against DDPH was evaluated using methods previously described by Manhtal et al. (2019), Ulewicz-Magulska Wesolowki (2019) and Chaves et al., 2020).

Briefly, test samples (2 mg dissolved in 2 ml methanol) and diluted in two fold dilution to have respective stock solution of 1 mg/ml and were diluted in twofold dilution with the same solvent to have a series of test concentrations from 20 to 0.1 µg/ml. 1 ml each test dilution sample was mixed with 1 ml methanol solution DDPH 0.4M and the mixture was nicely or gently mixed and left in obscurity for 16 minutes before the measure of absorbance on a spectrophotometer PerlinElmer Lambda 365 (USA) at 517 nm. DDPH 0.4M MeOH solution was used as negative control. The effect of test sample on DDPH was calculated using the following formula:

$$\% \text{ Inhibition of DPPH activity} = \frac{\text{AbsNC - AbsTS}}{\text{AbsNC}} \times 100$$

Where AbsNC is the absorbance of DPPH 0.4M MeOH solution and AbsTS the absorbance of test sample. The inhibition 50 (IC_{50}) of DPPH activity by each test sample was derived from linear courbes concentrations-responses (n=3).

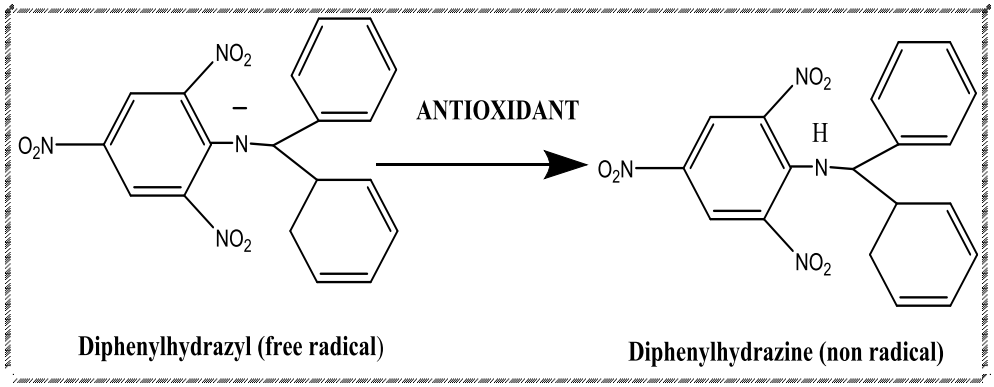

Figure 3A: Reduction of DPPH in the presence of antioxidant substance (Kedare and Singh., 2011).

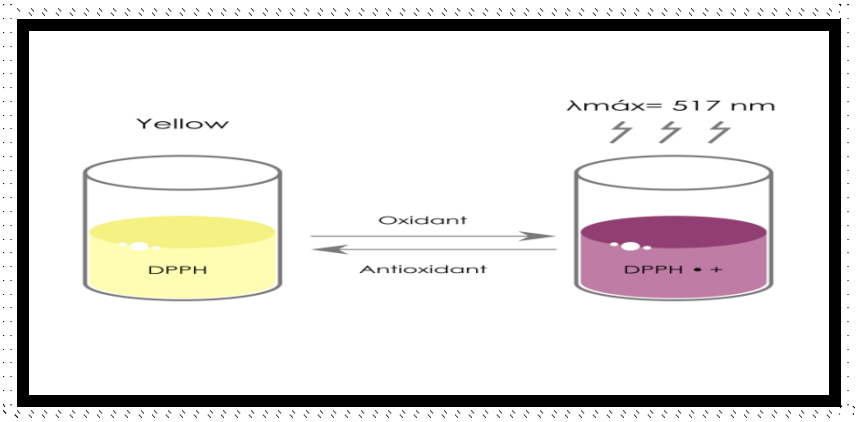

Figure 3B: Reactivity of DPPH radical with an antioxidant substance (Kedare and Singh, 2011).

2.4.2. Determination of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate)

Methods used were previously described by de Vargas et al., (2016), Labiad et al., (2017) and Chaves et al., (2020) based on the oxidation of ABTS were used. The oxidated ABTS solution is prepared by reaction of 2 mM ABTS in deionized water with potassium persulfate ($K_2S_2O_8$) kept in obscurity for 4 h. Before use, ABTS solution is diluted with phosphate sodic tampon (0.1M, pH 7.4) to have an absorbance of 0.750 at 734 nm. Test samples (2 mg dissolved in 2 ml methanol) to have respective stock solution of 1 mg/ml and were treated as described above to have a series of test concentrations from 20 to 0.1 µg/ml. After, 1 ml ABTS solution was added to 1 ml of test dilution sample and mixed gently. ABTS solution was used as negative control. Absorbances were measured on a spectrophotometer Perlin-Elmer Lambda at 734 nm and inhibition percentages calculated as:

$$\% \text{ Inhibition of ABTS activity} = \frac{\text{AbsNC - AbsTS}}{\text{AbsNC}} \times 100$$

Where AbsNC was the absorbance of negative control and AbsTS the absorbance of tested sample.

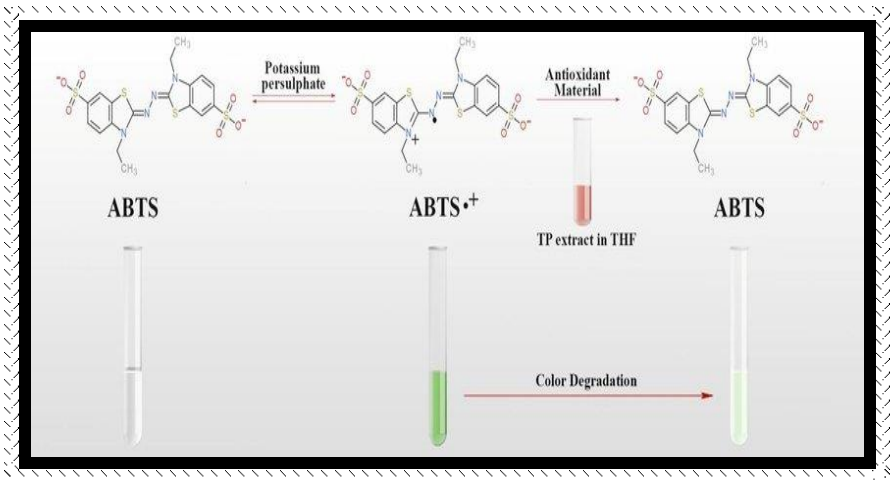

Figure 4: Reduction of ABTS and reaction of the formed radical with antioxidant material.

The inhibition concentration 50 (IC_{50}) ABTS activity by each sample was derived from linear courbes concentrations-responses (n=3).

2.4.3. Determination superoxide anions

For this, 2 mg of each test sample were dissolved in 2 ml MeOH to have respective stocks solution of 1 mg/ml They were diluted in twofold dilutions to have a series of test concentrations ranging from 20 à 0.1 µg/ml. The test was carried out in microplate titers with 6 holes. Each hole contained a known concentration of test dilution sample mixed with 250 µM nitro bleu tetrazolium (NBT, 100 µL) and 390 µM NADH (100 µL). Absorbances were recorded on the same spectrophotometer at 560 nm (de Vargas et al., (2016) and the inhibition of superoxide anions activity was calculated using the above formula. IC_{50} of each tested sample was derived as described above (Chavan et al., (2018, Wetchakul et al., 2022).

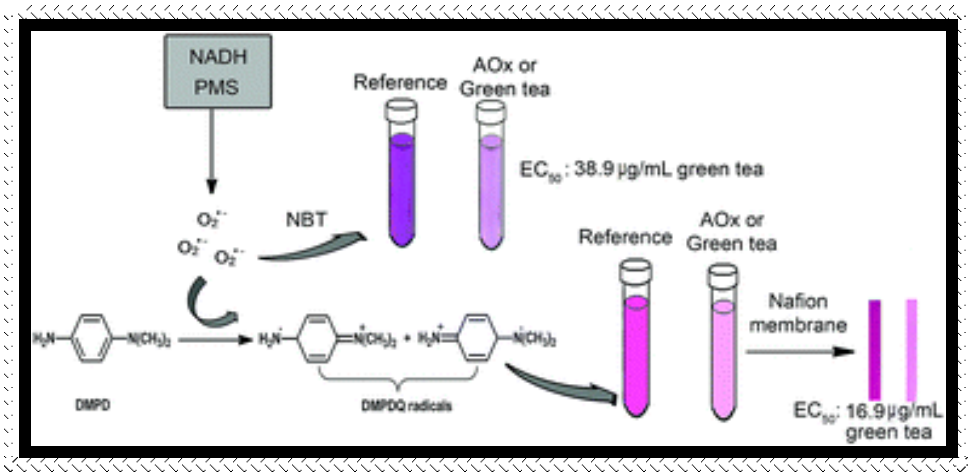

Figure 5: Superoxide anion and its reactivity.

This activity was evaluated using methods proposed by (Pleh1 et al., 2021, Richards and Chaurasia, 2022). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 1 mL of different concentrations (1-20 µg/mL) of Ascorbic acid as positive control and test samples prepared as described above, were separately added to 1 mL hydrogen peroxide solution (0.6 mL, 40 mM) and mixed gently. The absorbance of test tubes was recorded at 240 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide on the same spectrophotometer. The hydrogen peroxide percentage scavenging activity can be calculated using the formula above. In all test, lower absorbance of the reaction mixture indicated higher free radical-scavenging and Ascorbic acid (Vitamin C) was used as reference antioxidant product.

2.4.3. Determination of hydroxyl ($HO^{\cdot}$)

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium by methods proposed by Lalhminglui et al, (2018) and Khan et al., (2023). The reaction mixture containing $FeCl_3$ (100 mM), EDTA (1 mM), H_2O_2 (1 mM) and 2-deoxy-D-ribose (2.8 mM) were mixed with or without test sample dilutions of various concentrations (1-20 µg/mL) prepared as described above in 1 mL final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 h at 37°C. The mixture was heated in water bath for 15 min followed by the addition of 1 mL each of TCA (trichoro acetic acid) (2.8%) and TBA (thiobarbituric acid) (0.5% TBA) in 0.025 M NaOH containing 0.02% (Butylated hydroxyl-anisole acid (BHA). Finally the reaction mixture was cooled on ice and.

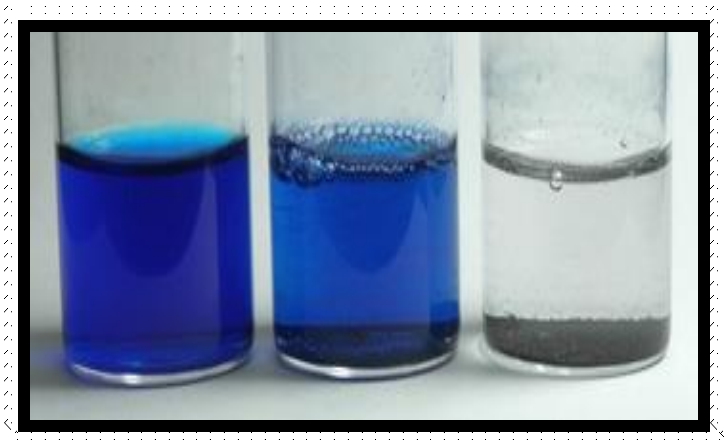

Figure 6. Reactivity of hydroxyl radical with an antioxidant substance.

Centrifuged at 5000 rpm for 15 min. 1 mL of this reaction mixture solution was mixed with test sample dilutions (1-20 µg/mL) and gently mixed. Absorbances were measured at 532 nm on the same spectrophotometer. All readings were corrected for any interference from color of the tested sample or antioxidant by including appropriate controls. The negative control without any antioxidant was considered 100% deoxyribose oxidation. The percentage inhibitions of hydroxyl radical scavenging activity of test samples can be determined in comparison with negative control using the above formula.

2.4.5. Assay of hydrogen peroxide scavenging activity

This activity was evaluated using methods proposed by (Pleh1 et al., 2021, Richards and Chaurasia, 2022). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 1 mL of different concentrations (1-20 µg/mL) of Ascorbic acid as positive control and test samples prepared as described above, were separately added to 1 mL hydrogen peroxide solution (0.6 mL, 40 mM) and mixed gently. The absorbance of test tubes was recorded at 240 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide on the same spectrophotometer. The hydrogen peroxide percentage scavenging activity can be calculated using the above formula. In all test, lower absorbance of the reaction mixture indicated higher free radical-scavenging and Ascorbic acid (Vitamin C) was used as reference antioxidant product.

2.5. Estimation total phenolic compounds

As flavonoids were not detected in A. congensis root ark, only the quantity of total phenolic compounds in aqueous and methanol 80% extracts was determined using Folin-Ciocalteu (FC)'s reagent by the methods described by Yandag et al., (2019)) and Dom et al., (2020). Gallic acid was used as standard (5-25 µg/ml). Different test concentrations of standard and extract (1-20 µg/ml) were introduced separately in different tubes mixed with 1 ml de FC (1 :1 dilution). 5 minutes after, 2 ml sodium carbonate de sodium ($CaCO_3$, 7.5%) were added, mixed carefully and left in obscurity for 15 minutes. After this period of incubation, absorbances were recorded on a spectrophotometer Perlin-Elmer Lambda at 760 nm. The quantity of total phenolic compounds was expressed in term gallic acid equivalent per 100 g of dried extract.

3. RESULTS AND DISCUSSION

3.1. Antispasmodic effects of extracts and fractions from *A. congensis* root bark

Following criteria were adopted for good understanding and interpretation of reported results: 90 < % IAD ≤ 100%: pronounced activity, 70 < % IAD ≤ 80%: good activity, 60 < % IAD ≤ 70: moderate activity, 50 < % IAD ≤ 60 % : weak activity, % IAD < 50%: inactive.

IAD: inhibition contractions of agonist acethylcholine and depolarization solution rich in KCl.

Results in Table 1 revealed that aqueous AC-1 extract, tested at 40 µg/ml in organ bath inhibited significantly contractions induced by acethylcholine (ACh) and depolarization solution rich in KCL (DSR-KCl producing 83.12±0.12 and 80.34±0.13% with effective doses (ED_{50}) values of 7.26±0.06 and 8.76±0.05 µg/ml respectively.

Chloroform AC-1.1 soluble fraction rich in steroids and terpenoids produced 68.78±0.13 and 65.85±0.12% inhibitions of contractions i induced by ACh and DSR-KCl with ED_{50} values of 12.36±0.08 and 14.23±0.13 µg/ml respectively, while ethylacetate AC-1.2 soluble fraction rich in flavonoids and residual aqueous AC-1.4 soluble fraction rich in other polyphenolic compounds than flavonoids exerted this inhibitory effect by producing 78.85±0.14 and 76.56±.0.14%, and 75.65±0.13 and 73.68±0.15% inhibitions of contractions induced by thecagonist ACh and DSR-KCl on isolated guinea pig ileum, with ED_{50} values of 8.56±0.05 and 9.12±0.07 and 9.02±0.08a and 10.56±0.09 µg/ml µg/ml respectively, as good activity.

n-butanol AC-1.3 rich in saponins produced the inhibition at percentages of 66.85±0.09 and 64.85±0.12% for ED_{50} values of 13.65±0.11 and 14.03±0.10 µg/ml respectively as moderate activity on Ach and DSR-KCl-induced contraction on isolated organ.

80% methanol AC-2 and total alkaloids AC-3 extracts produced 84.78±0.08 and 86.02±0.11%.

Table 1: Effects of extracts and soluble fractions on acetylcholine (ACh) and depolarisation solution rich in KCl (DSR-KCl)-induced contractions on isolated guinea-pig ileum.

Extracts/Fractions code	% inhibition Ach contractions (IAD)	ED_{50} (µg/ml)	% Inhibition DSR-KCl s contractions (IAD)	ED_{50} (µg/ml)
AC-1	83.12±0.12	7.26±0.06	80.34±0.13	8.76±0.05
AC-1.1	68.78±0.13	12.36±0.08	65.85±0.12	14.23±.013
AC-1.2	78.85±0.14	8.56±0.05	76.56±0.14	9.12±0.07
AC-1.3	66.85±0.09	13.65±0.11	64.85±0.12	14.03±0.010
AC-1.4	75.65±0.13	9.02±0.08	73.68±0.15	10.56±0.9
AC-2	84.78±0.8	5.62±0.05	82.64±0.11	6.52±0.05
AC-3	86.02±0.11	3.25±0.04	84.24±0.14	4.15±0.04

Atropine sulphate	100.00±0.10	3.25±0.04	0.00±0.00	0.00±0.00
Papaverine 2HCl	100.00±0.10	2.15±0.03	98.92±0.08	3.65±0.03

AC-1: aqueous extract, AC-1.1 to 1.4 chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions, AC-1: aqueous extract, AC-2: 80% methanol extract, AC-3: total alkaloids extract.

and 86.02±0.11 and 84.24±0.14% inhibition of contractions induced by ACh and DSR-KCl with ED_{50} values of 5.62±0.02 and 3.25±0.01 µg/ml, and 3.25±0.04 and 4.15±0.04 µg/ml respectively as good activity.

In comparison of spamolytic activity of these samples from *A. congensis* root bark, it was remarked that soluble fractions exhibited weak activity compared to parent aqueous AC-1 extract and suggested that they can act in synergistic manner to restore the high activity showed by the parent extract (Table 1). In addition, 80% methanol extract AC-1 displayed high activity compared to aqueous AC-1 extract and this may be due to the influence of the nature of extractive solvent (80% methanol versus water) while ethylacetate AC-1.2 soluble fraction displayed high activity compared to remaining soluble fractions AC-1.1, 1.3 and 1.4 due probably to the nature of constituents present in these soluble fractions. The antispasmodic activity developed by all samples from *A. congensis* was low likened to Atropine and Papaverine dihydrochloride used and reference antispasmodique drugs (Table 1).

There was significant difference in amplitudes between the contractions induced by ACh and DSR-KCl alone and in the presence of tested samples from *A. congensis* root bark leading to the inhibition of their action since the amplitude induced by ACh and DSR-KCl alone was high compared to that in presence of test samples which was short. This effect suggested that these samples may have act through the inhibition of muscarinic receptors M_2 and M_3, achieved by muscarinic receptor antagonists or antimuscarinic agents, blocked the activity of muscarinic acetylcholine receptors (mAChRs), reducing parasympathetic nervous system activation and counteracting "rest-and-digest" responses. Muscarinic antagonists, also known as anticholinergics, blocked muscarinic cholinergic receptors, producing mydriasis and bronchodilation, increasing heart rate and inhibiting secretions.
(https://www.google.com/search?q=inhibition+of+muscarinic+receptor&rlz=1C1GCEA_enBE1140BE1140&oq=inhibition+of+muscarinic+receptor&gs_lcrp=egzjahjvbwuybggaeeuyotihcaeqabiabdiicaiqabgwgb4ycagdeaayfhge0gekmjc3mzdqmgoxnagcclacafefj77b3vkhtv8&sourceid=chrome&ie=UTF-8, 2024)

All test samples produced sponptaneously good relaxation of isolated guinea-pig ileum when tested at 40 µg in organ batch. It had been well demonstrated that the spontaneous contractions of the intestinal smooth muscle were due to cycles of depolarization involving a fast influx of Ca^{2+} which increased the cystosolic Ca^{2+} and subsequently, activated the contractile elements. The increase in the cystosolic Ca^{2+} can occur either via influx of the ion through the voltage-operated calcium channel (VOCC) or release of calcium from sarcoplasmic reticulum. The frequency of the contractions was not altered by any of the test substances suggesting that they do not modify the frequency of the spontaneous depolarization from the pacemaker cells (Yan et al., 2020).

Aqueous AC-1 extract and its soluble fractions as well as 80% methanol and total alkaloids extracts caused inhibition of spontaneous contractions in isolated guinea-pig ileum induced by ACh and DSR-KCl tested at 40 µg/ml in organ batch, showing thus their spasmolytic or antispasmodic effect. Extracts and soluble fractions from *A.congensis* root bark exhibited low spasmolytic effect compared to reference antispasmodic products Atropine sulfate and Papaverine dichorhydrate as already mentioned above, as the first one inhibited only contraction induced by ACh and the second inhibited both contractions induced by ACh and DSR-KCl. Based on this observation, these samples from A. congensis root bark were considered to possess Papaverine-like effects.

Figures 7 and 8 showed that tested aqueous AC-1, 80% methanol AC-2 and total alkaloids AC-3 extracts inhibited contractions induced by acetylcholine (ACh) and DSR-KCl on isolated guinea-pig ileum in concentrations-dependent manner from 5 to 50 µg/ml. The inhibition percentages were low with the test carried with low concentraions of 5 to 30 ug/ml at which contractions induced by ACh had values from 10.12 to 60.76% for aqueous extract AC-1 extract, from 13.56 to 81.37% for methanol 80% AC-2 extract and from 17.12 to 82.76% for total alkaloid AC-3 extract. This ecreased percentage d knew significant increase related to the use of high concentrations of 40 and 50 µg/ml and reached high values of 83.12 and 103.53 for aqueous C-1 extract, of 84.78 AND 101.05 for methanol 80% AC-2b extract and of 86.02 and 107.52% for total alkaloids C-3 extract.

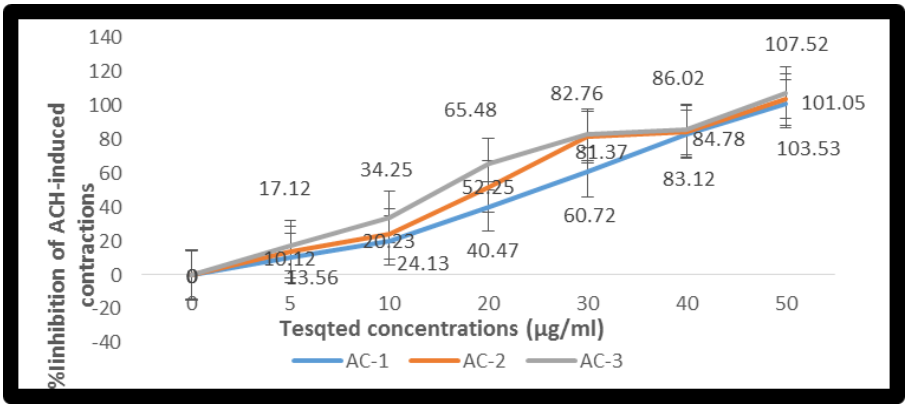

Figure 7: Inhibition percentages of ACh-induced contractions on isolated guinea-pig ileum by aqueous AC-1, 80% methanol AC-2 and total alkaloids AC-3 extracts.

The impact of these extract on DSR-KCl-induced contractions on isolated organ revealed that tested at low concentrations from 5 to 30 µg/ml, they produced inhibition percentages from

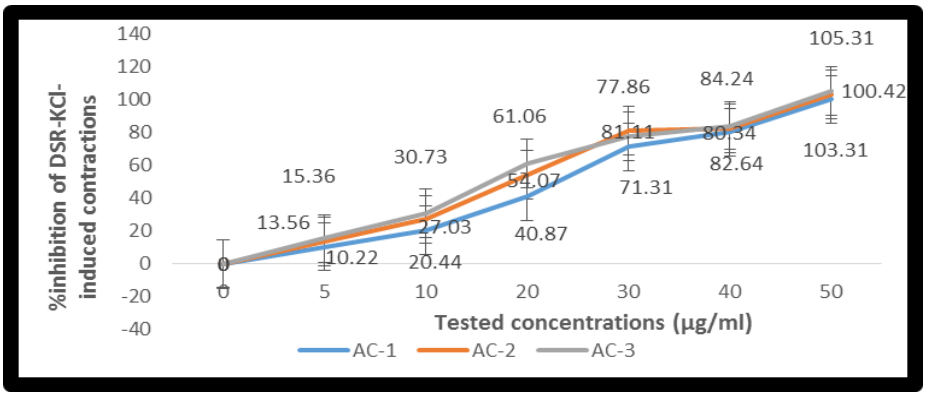

Figure 8.Inhibition percentagess of DSR-KCl induced contractions on isolated guinea-pig ileum by aqueous AC-1, 80% methanol AC-2 and total alkaloids AC-3 extracts.

10.22 to 7131%, 13.56 to 81.11% and 15.36 to 77.86% attributable to aqueous C-1 extract, methanol 80% AC-2 extract and total alkaloids AC-3 extract respectively. In both tests, total alkaloids AC-1 extract exhibited high antispasmodic activity likened to aqueous AC-1 and methanol 80% AC-2 extracts respectively.

Sometimes, the paralysis of isolated organ can occur at this highest concentrations, fortunately, this was not observed during our experiment.

3.2. Antispasmodic activity of isolated compounds

Results revealed that boonein inhibited ACh and DSR-KCl-induced contractions on isolated organ by producing 72.68±0.11 and 70.25±0.12% with effective doses 50 (ED_{50}) of 12.65±0.11 and 14.26±0.08 µg/ml as good activity. Echitamine and 6,7 Seco-angustilobine exerted the

Table 2: Effects of isolated compounds on ACh and DSR-KCl-induced contractions on isolated guinea-pig ileum.

Compounds	% IADCh	ED_{50} (µg/ml)	% ICADSR-KCl	ED_{50} (µg/ml)
Boonein	72.68±0.11	12.65±0.11	70.25±0.12	14.26±0.08
Echitamine	82.65±0.12	6.54±004	80.48±0.14	7.82±0.06
6.7 Seco-angustilobine	80.65±0.14	8.25±0.05	78.72±0.13	9.56±0.09
β-Amyrine	70.56±0.12	13.25±0.07	68.65±0.11	15.23±0.08
Atropine SO_4	100.00±0.00	3.25±0.05	0.00±0.00	0.00±0.00
Papaverine 2HCl	100.00±0.00	2.15±0.03	98.92±0.10	3.65±0.04

% IADCh: inhibition percentages contraction induced by acethylcholine, % ICDSR-K: inhibition percentages contraction induced by DSR-KCl.

Same effect by producing 82.65±0.11 and 80.65±0.14% against ACh and DSR-KCl with ED_{50} values of 6.54±0.04 and 7.82±0.05 µg/ml respectively, and 80.65±0.11 and 78.72±0.13% towards ACh and DSR-KCl induced contractions on isolated guinea-pig ileum

with ED_{50} values of 8.25±0.05 and 9.56±0.09 µg/ml respectively as good activity. The terpene β-amyrine presented inhibition on ACh and DSR-KCl-induced contractions by 70.56±0.12 and 68.65±0.11% with ED_{50} values of 13.25±0.07 and 15.23±0.08 µg/ml as moderate activity. All isolated compounds from *A. congensis* root bark exhibited weak antispasmodic activity compared to Atropine sulphate and Papaverine 2HCl and possessed Papaverine-like effects for the same reasons evoked above (Table 2).

In the comparison of spasmolytic activity exerted by extracts and soluble fractions, it was found that the activity exerted by all extracts was significantly high likened to soluble fractions and isolated compounds with significant difference ($p< 0.05$). The activity showed by isolated compounds was high compared to that of soluble fractions chloroform AC-1.1 and *n*- butanol AC-1.3 and Bonein showed weak activity confronted to soluble fraction ethylacetate AC-1.2 and residual aqueous phase AC-1.4 and high activity compared to soluble fraction AC-1.1 and *n*-butanol AC-1.3 on ACh and DSR-KCl respectively while beta-amyrine exhibited high activity on ACh and DSKCl induced contractions compared to chloroform AC-1.1 and n-butanol AC-1.3 (Table 1). Aqueous AC-1 extracta and Echitamine exerted similar activity against DSR-KCl-induced contraction on isolated organ (Tables 1 and 2).

Figures 9 and 10 showed the inhibition of contractions induced by ACh and DSR-KCl on isolated guinea-pig ileum by Boonein, Echitamine and 6,7-Secoangustilobine tested at different concentrations from 5 to 50 µg/ml. Results indicated that theses isolated compounds inhibited these contractions in concentrations-dependent manner (Fig. 4 and 5).

Tested at low concentrations from 5 to 20 µg/ml, they produced low inhibition percentages from 9.10 to 42.12% on ACh-induced contractions and from 8.81 to 42.41 on DSR-KCl This percentage increase significantly with the use and of highest concentrations of 30 and 40 µg/l showing inhibition percentages from 54.51 to 84.24 and from 60.48 to 84.78, against ACh and DRS-KCl respectively. At the highest tested concentration of 50 µg/ml, Echitamine produced 105.30 and 106.01% inhibition of contractions-induced by Ach agonist and DSR-KCl, Booenein, Echitamine and 6,7-Secoangustiobine showed 100.51 and 106.01, 90.85 and 87.82, 100.51 and 100.81% inhibition of contractions induced by ACh and DSK-KCl respectively. Echitamine was the most active and Boenin was the less active towards ACh and DSR-KCl-induced contractions on isolated organ (Fig. 4 and 5).

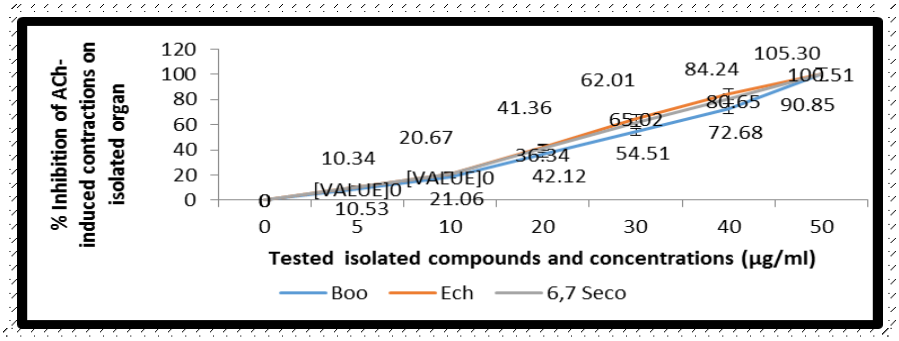

Figure 9: Inhibition percentages of Boonein (Boo), Echitamine (Ech) and 6,7 Secoangustilobine (6,7 Seco) on ACh-induced contractions on isolated organ.

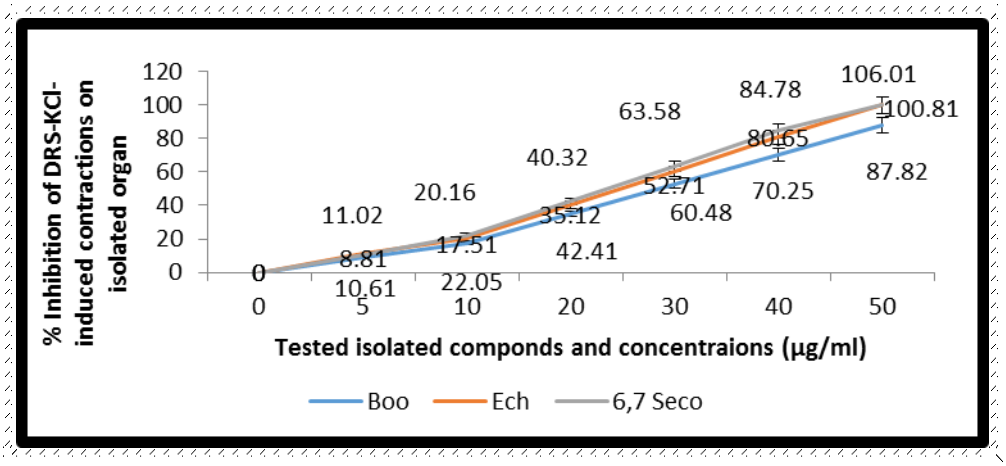

Figure 10: Inhibition percentagess of Boonein (Boo), Echitamine (Ech) and 6,7 Secoangustilobine (6,7 Seco) on DSR-KCl-induced contractions on isolated organ.

Results from the spasmolytic effects showed by extracts, soluble fractions and isolated compounds from *A. congensis* root bark was manifested their effects on muscarinic receptors as also previously reported by Devi et al., (2011) for extracts of *Cymbopogon citratus* on isolated rabbit ileum. This finding suggested that high and low concentrations of these samples and Atropine may inhibit muscarinic receptors and decrease intestinal contractions as it was reported that the inhibition of these receptors in gastro-intestinal disorders. These samples from *A. congensis* root bark can lead to the relaxation of intestinal smooth muscles. Our observation was in good agreement with Tambuli et al., (2022), _Piqué-Borrà et al., (2025).

In addition, it can be assumed that tested concentration of *A. congensis* samples blocked M_2 and M_3 receptors to inhibit induced intestinal contractions since these receptors were known to be mainly responsible for intestinal contractions exhibited mainly by acetylcholine. Inhibition of muscarinic receptors, specifically M2 and M3, can be achieved through various mechanisms, with M3 receptor antagonists like tiotropium being used to treat conditions like asthma and overactive bladder, while M2 receptor antagonists can also have therapeutic effects. In smooth muscle, M3 receptors mediated phosphoinositide hydrolysis and Ca^{2+} mobilization, whereas M2 receptors mediated an inhibition of cAMP accumulation. The inhibitory effect of the M2 receptor on cAMP levels suggested an indirect role for this receptor, namely, an inhibition of the relaxant action of cAMP-stimulating agents. Functionally, M_3 receptors promoted Cl^- secretion, whereas M_1 receptors may act in an inhibitory manner. It was also reported that these both receptors were present in isolated preparation of intestinal smooth muscles (Ehlert et al;, 1999, Rauf et al., 2021, Uwada et al., 2023).

It was also observed that spasmolytic activity of medicinal plant extracts and fractions was usually mediated through calcium channel blockade (Gilani et al., 2006, 2010). To evaluate whether the antispasmodic activity of the plant part studied was also mediated through calcium channel blockade (CCB), high K^+ 100 mM) as KCl was used to depolarize the preparations. Thus, high K^+ (> 30 mM) was already known to cause smooth contractions through opening of voltage-dependent L-Type Ca^{2+} channels allowing influx of extracellular Ca^{2+} causing a contractile effect. A substance causing inhibition of high K^+ induced contractions, was considered as a blocker of Ca^{2+} influx or calcium channel blocker (CCB) (Gilani et al., 2010, Ramachandran et al., 2011, Asifa et al., 2017, Okoye et al. 2023).

Calcium channel blockers (CCB), calcium channel antagonists or calcium antagonists are a group of medications that disrupt the movement of calcium (Ca^{2+}) through calcium channels. Calcium channel blockers are used as antihypertensive drugs, i.e., as medications to decrease blood pressure in patients with hypertension. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients.[Calcium channel blockers are also frequently used to alter heart rate (especially from atrial fibrillation), to prevent peripheral and cerebral vasospasm, and to reduce chest pain caused by angina pectoris (Anonyme, 2024).

Calcium channel blockers (CCBs), also known as calcium channel antagonists, have been approved by the US Food and Drug Administration (FDA) and are widely used to treat various conditions such as hypertension, coronary heart disease, and chronic stable angina. However, despite their widespread use, this class of cardiovascular drugs is one of the primary contributors to drug-related fatalities (McKeever et al., 2024).

Correlating search had shown that K^+ at high concentration greater than 30 mM, was able or proficient of opening voltage-dependent calcium channels (VDCCs) resulting in causing the smooth muscle contractions and fundamentally or essentially a contractile action by influx of extracellular calcium depicting inhibition of potassium-induced contractions (Aleem and Jambaz., 2018; Okoye et al., 2023). Periodic depolarization and re-polarization of the tissues were due to the influx of calcium into sarcoplasmic reticulum through voltage-dependent calcium channel.

They were known to be responsible for spontaneous intestinal responses or produced spontaneous intestinal movements and depolarizing peaks constituted a result because of quick influx Ca^{2+} via voltage-dependent L-type Ca^{2+} channels (Asifa et al., 2017; Vadivel et al., 2017, Rong-Shan et al., 2020). A perception can be made that spasmolytic effect showed by samples from *A. congensis* root bark could be the manifestation of the calcium channel blockers (CCB) as also previously reported Naz et al., (2016) Asifa et al., (2017) for extracts from *Polypodium vulgare* L. and *Spinacia oleraceae* L. respectively.

To confirm the presence of Ca^{2+} channels blocking effects, and the spasmolytic activity of samples from *A. congenesis* root bark, was mediated through Ca^{2+} channel blockade (CCB), high concentration of K+ (100 mM) was used to depolarize the preparation instead 30 mM (Asifa et al, 217) as also previously reported by Cimanga et al., (2024) as mentioned above.

In these conditions, extracts, soluble fractions and isolated compounds from *A. congensis* root bark, were analyzed on high K^+ (100 mM) induced contractions on isolated guinea-pig ileum and results showed that these samples inhibited concentrations induced by high K^+ (100 mM) in depolarization solution rich in KCl indicating antispasmodic effects of the tested samples.

Extracts, fractions and isolated compounds from *A. congensis* root bark, relaxed thus the high K^+ (100 mM) induced contractions, similar to Verpamil, a known calcium channel blocker as also reported by Ramachandran et al., (2011) and may be due to the calcium channel blockade as also previously reported by Ahmad et al., (2012) for extracts and fractions from *Euphorbia granulate*.

The calcium channel blocking effects observed was due to the presence of different phytochemical groups such as, flavonoids, steroids, terpenoids, saponins, tannins, alkaloids and polysaccharides identified previously in this medicinal plant part (Lumpu et al., 2013; Cimanga et al., 2016)**.** This finding indicated the involvement of Ca^{2+} channel blocking activity in spasmolytic effect because any agent inhibiting high K^+ concentrations inducing contractions was denoted calcium channel blocker (CCB) as already mentioned above (Asifa et al., 2017; Okoye et al., 223, Cimanga et al., 2024).

The presence of Ca^{2+} antagonist constituents was further confirmed when pre-treatment of the tissue with samples from *A. congensis* root bark shifted the Ca^{2+} tested samples to the right as also reported by Gilani et al., (2010). It was also known that calcium antagonists constituted an important therapeutic group with the common characteristic as their dose-dependent inhibition of slow entry of Ca^{2+} and their ability for reversal this effect by Ca^{2+} (Gilani et al., 2010).

Reduction of KCl -induced contractions suggested that samples from *A. congensis* root bark inhibited the influx of Ca^{2+}. KCl did'nt act trough a receptor and was known to elevate Ca^{2+} concentration via calcium channels as also reported by Nwaiwu and Nwaiwu., (2015). These samples probably reduced the contractions of isolated guinea-pig ileum via blocking these channels. In KCl-induced contractions, the voltage dependent calcium channels (VDCCs) were involved and the existence of L-Type VDCC in the rat ileum had been reported. It was suggested that those substances that inhibited the KCl-induced contractions acted via blocking these channels and were called CCB blockers (Nwaiwu and Nwaiwu, 2015; Asifa et al., 2017). Thus extracts and soluble fractions from *A. congenesis* root bark can be considered as CCB agent mediating putatively through calcium channel blockade.

3.2. Antioxidant activity of extracts and fractions from *A. congensis* root bark

Following criteria were adopted for good interpretation of reported results: $IC_{50} \leq 10$ µg/ml: pronounced activity, $10 < IC_{50} \leq 20$ µg/ml: good activity, $20 < IC_{50} \leq 30$ µg/ml: moderate activity, $30 < IC_{50} \leq 50$ µg/ml: weak activity, $IC_{50} > 50$ µg/ml: inactive.

The plant extract, soluble fractions and constituents possessing antioxidant properties acted as antioxidants and free radical scavengers by converting them to less reactive species. Antioxidants are a class of chemical substances naturally found in foods and can prevent or reduce the oxidative stress of the physiological systems (Laksmibai and Amirtham, 2018).

Table 3 llustrated the results of single oral administration of *A. congensis* root bark aqueous extract at different doses (100, 200, and 500 mg/kg) in rats. Observations for 28 days showed no signs of toxicity (vomiting, nausea, tremors, sleep, aggressiveness, diarrhea, and mobility). In addition, this administration did not cause the death of the rats in all of the batches treated.

Table 3: Effects of *A. congensis* root bark aqueous extract on General Behavior in the Rat[a]

Sigyns	Oral doses		
	500 mg/kg	1000 mg/kg	5000 mg/kg
Number of animals	5	5	5
Aggressiveness	N	N	N
Diarrhea	N	N	N
Weakness	A	A	A
Mobility	N	N	N
Nausea	N	N	N
Sleep	N	N	N
Tremor	N	N	N
Vigilance	N	N	N
Vomiting	N	N	N
Number of dead	0	0	0

N: Normal, A: absent compared to negative control.

According to Figure 11, the examination of the weight evolution of the rats showed that the groups treated with the different doses (100, 200, 600 mg/kg) gradually gained weight during the 14 days likened to negative control group (Fig. 6).

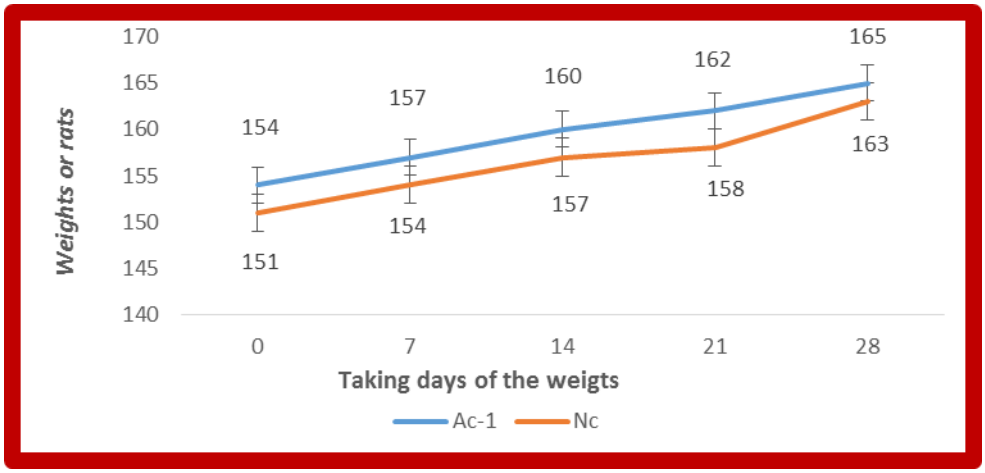

Figure 11: Variation of weight rats after oral administration once of the highest oral dose of 5000 mg/kg bw.

Fruits and vegetables were known as the most common sources of antioxidants and regular consumption of them will decrease the chances of the occurring chronic and degenerative diseases. Natural antioxidants can be found in all plant parts and they include carotenoids, vitamins, phenols such as flavonoids, tannins and other (Anuj et al., 2016; Rajopal et al., 2019). The body constantly produced free radicals due to regular use of oxygen. These free radicals were responsible for the cell damages in body and contributed to various kinds of health problems (Mamta et al., 2013; Rajagopal et al., 2019).

In the present study, results in Table 4 revealed that aqueous AC-1 extract inhibited the activities on all selected reactive oxygen species (ROS) with IC_{50} values ranging from 5.00±0.03 to 11.07±0.04 µg/ml. In fact, this extract inhibited the activity of DPH, ABTS, $O^{2\bullet-}$, and $HO^{\bullet}$ radicals with IC_{50} values of 4.15±0.04, 5.00±0.05, 7.05±0.05 and 9.15±0.06 µg/ml respectively as pronounced activity and against H_2O_2 radical with IC_{50} value of 11.07±0.10 µg/ml as good activity.

Soluble fractions chloroform AC-1.1 and ethylacetate AC-1.2 soluble reacted in the same manner by causing the inhibition of the activities of DPPH with IC_{50} values of 8.36±0.05 and 7.05±0.06 µg/ml and against ABTS with IC_{50} values of 10.05±004 and 8.02±0.06 as good and pronounced activity respectively, against $O^{2\bullet-}$ and $HO^{\bullet}$ with IC_{50} values of 12.17±0.07 and 10.05±0.06, and 13.25±0.04 and 11.25±0.09 µg/ml respectively as good activity and against H_2O_2 whith IC_{50} values of 14.05±0.12 and 13.24±0.11 as also good activity.

Table 4: Effects of extracts and soluble fractions on reactive oxygen species (ROS), IC_{50}, µg/ml.

Sample codes	DPPH	ABTS	$O^{2\bullet-}$	$HO^{\bullet}$	H_2O_2
AC-1	4.15±0.04	5.00±0.03	7.05±0.06	9.15±0.06	11.07±0.08
AC-1.1	8.36±0.05	10.05±0.04	12.17±0.10	13.25±0.10	14.05±0.11
AC-1.2	7.05±0.06	8.02±0.06	10.05±0.09	11.25±0.09	13.24±0.10
AC-1.3	11.31±0.7	13.21±0.10	15.00±0.11	12.15±0.11	15.32±0.12
AC-1.4	5.22±0.05	6.54±0.08	8.35±0.07	10.51±0.11	11.05±0.09
AC-2	3.45±0.4	4.02±0.03	5.22±0.06	7.10±0.05	8.36±0.07
Total phenolic compounds	3.23±0.04	4.00±0.04	5.02±0.07	5.83±0.04	7.55±0.05
Quercetin	3.25±0.04	4.25±0.05	8.56±0.07	10.00±0.08	13.02±0.09
Gallic acid	2.15±0.03	3.02±0.03	6.85±0.05	8.74±0.06	8.03±0.06

DPPH : 1,1-diphenyl-2-picryl-hydrazyl**)**, ABTS : 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate, $O_2\bullet-$: superoxide anions, $HO\bullet$: hydroxyl, H_2O_2: hydrogen peroxide.

n-butanol AC-1.3 and residual aqueous AC-1.4 soluble fractions exerted the same effect by producing IC_{50} values of 11.31±006 µg/ml as good activity and 5.22±0.04 µg/ml as pronounced activity towards DPPH respectively, 13.21±0.07 µg/ml as good activity and 6. 54±0.04 µg/ml as pronounced activity towards ABTS, presented 15.00±0.08 µg/ml as good activity and 8.35±0.05 µg/ml as pronounced against $O^{2\bullet-}$ respectively, 12.15± 0.03 and 10.51±0.09 against $HO^{\bullet}$ and 15.32±0.12 and 11.05±0.10 µg/ml against H_2O_2 as good activity.

80% methanol AC-2 caused the inhibition activities of all selected ROS by producing IC_{50} values of 3.45±0.04 and 4.02 ±0.05 against DDPH and ABTS, 5.22±0.04 and 7.10±0.06 towards $O^{2\bullet-}$, $HO^{\bullet}$ and 8.36±0.07 µg/ml towards H_2O_2 radicals respectively as pronounced activity.

Total phenolic compounds exhibited also pronounced inhibition of activities of all selected ROS with IC_{50} values of 3.23±0.05, 4.00±0.04, 5.02±0.04, 5.83±0.04

and 7.55±0.04 µg/ml towards DPPH, ABTS, $O^{2\bullet-}$, $HO^{\bullet}$ and H_2O_2 radical respectively as pronounced activity.

In general, by producing antioxidant activity, all tested samples from *A. congensis* root bark exhibited interesting and appreciable free radical scavenging and antioxidant activities as also previously reported by Kozlowska et al., (2022) for the antioxidant of other extracts from selected plant material.

By comparison the activity of extracts and soluble fractions between them and to reference antioxidant products, firstly, it was perceived that methanol 80% AC-2 extract exhibited high activity compared to aqueous extract AC-1 and its soluble fraction with significant difference ($p < 0.05$) while aqueous extract AC-1 displayed prominent effect likened to its soluble fractions with remarkable difference ($p < 0.05$), suggesting that these last can react in synergistic manner to restore the prominent activity of the parent aqueous AC-1 extract. Secondly, it was observed that all extracts and soluble fractions showed low activity compared to reference antioxidant products (Table 4) with significant difference ($p < 0.05$), but total phenolic compounds showed s similar activity compared to Quercetin since no significant ($p > 0.05$) difference was deduced.

Figure 7 showed the free antioxidant and scavenging activities of aqueous extract AC-1 and 80% methanol AC-2, Quercetin and Gallic acid on DPPH activity. Results revealed that, at low tested concentrations of 2 and 4 µg/ml, aqueous extract AC-1 and methanol 80% AC-2 extract produced percentage inhibitions less from 24.11 to 96;95% while Gallic acid and Vitamin C fournished percentage inhibitions from 30.72 to 93.32 showed by (93.02%) Aqueous extract AC-1 was the most active and methanol 80% extract AC-2 was the less active (Fig. 7)

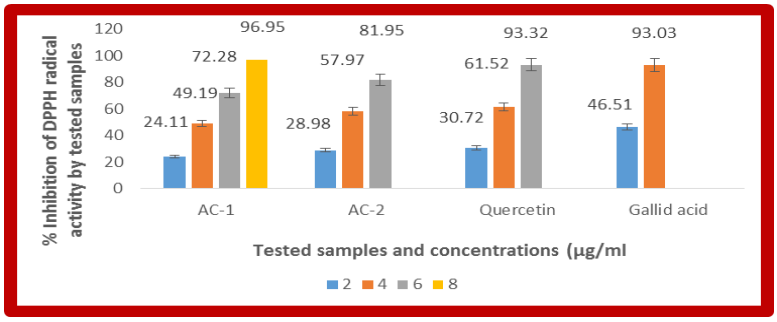

Figure 12: Antioxidant and free scavenging effects of aqueous extract AC-1 and 80% methanol extract AC-2 , Quercetin and Gallic acid on DPPH radical activity.

Figure 8 reported the antioxidant and free scavenging radical activities of aqueous aC-1 extract, 80% methanol AC-2 extract, Quercetin and Gallic acid on ABTS radical activity.

Results indicated that when tested at low concentrations of 2 and 4 µg/ml, aqueous AC-1 extract produced low inhibition percentages than 50% (Fig.), 80% methanol AC-2 extract and quercetin fournishes low concentration than 50% at 2 µg/ml and more than 50% at 4 µg/ml (Fig. 7);Tested at highest concentration of 6 and 8 µg/ml, aqueous AC-1 extract and 80% methanol AC-2 extract produced 70.87 and 80.67% inhibitions, quercetin and Gallic acid 92.65 and 91.87% with Gallic acid as the most active since it showed percentage inhibition at high percetange inhibition at low concentration of 4 µg/ml likened to other samples and the less active was methanol 80% AC-2 extract (Fig. 7)

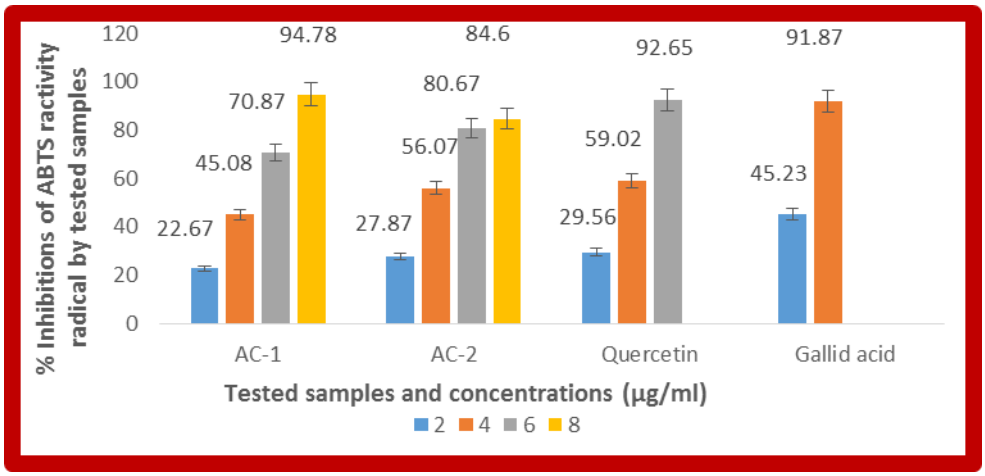

Figure 13: Antioxidant and free scavenging radical effects of aqueous extract AC-1 and 80% methanol extract AC-2, Quercetin and Gallic acid on ABTS radical activity.

Figure 9 showed the antioxidant and free scavenging activities of aqueous extract AC-1 and 80% methanol AC-2, Quercetin and Gallic acid on superoxide anions radical activity Results revealed that at low concentrations of 2 and 4 µg/ml, all test samples bended the activity of superoxide anions with inhibition percentages less than 50% (Fig. 6), except Gallic acid presenting 56.15% inhibitions at 2 µg/ml, Quercetin and Gallic acid producing 85.67 and 95.75% inhibition at 4 µg/ml and also methanol 80% AC-2 extract showing 42.55% inhibition.

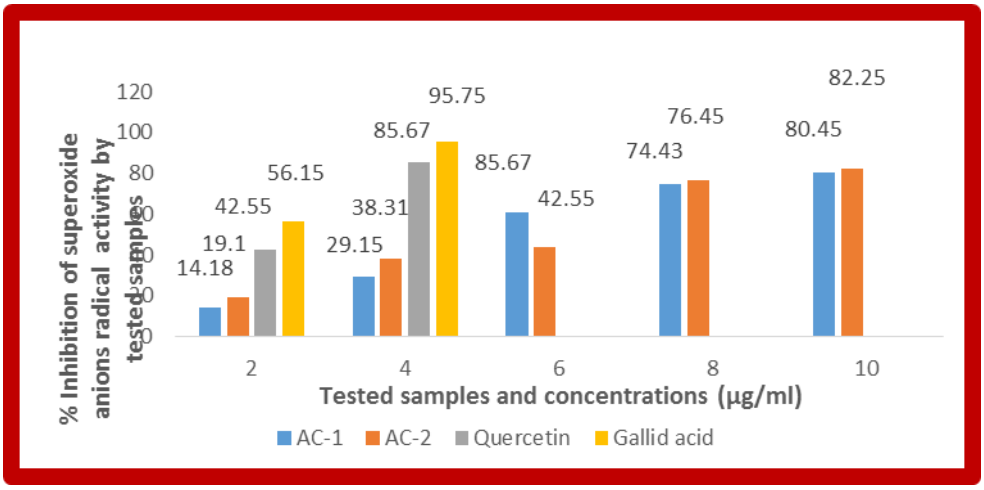

Figure 14: Free scavenging effects of aqueous extract AC-1 and 80% methanol extract AC-2, Quercetin and Gallic acid on superoxide anions (O^{2-}) radical activity.

at 6 µg/ml. Despite this effect, significant increase of this percentage was obtained with the use of high test concentrations from 6 to 10 µg/ml reaching inhibition percentages from 74.43 to 82.25% without forgotten that aqueous AC-1 extract generated 85.67% inhibition at 6 µg/ml. Gallic acid was the most active (Fig. 7). At 6, 8 and 10 µg/ml, Quercetin and Gallic acid produced percentage inhibitions 1002.54 and 105.07% respectively.

Figure 10 reported the antioxidant and free scavenging activities of aqueous extract AC-1 and 80% methanol AC-2, Quercetin and Gallic acid on hydroxyl radical activity. It was observed that aqueous and 80% methanol extracts AC-1 and AC-2 respectively and Quercetin showed inhibition percentages from 10.92 to 43.15% when tested at concentrations of 1 and 4 µg/ml respectively, except gallic acid showing 53.04% at 4 µg/ml (Fig. 10). Next, this percentage knew significant increase using high concentrations of 6 and 8 µg/ml at which they produced percentage inhibitions ranging between 57.61 and 92.23%. Gallic acid as the most active and Quercetin was the less active (Fig. 8).

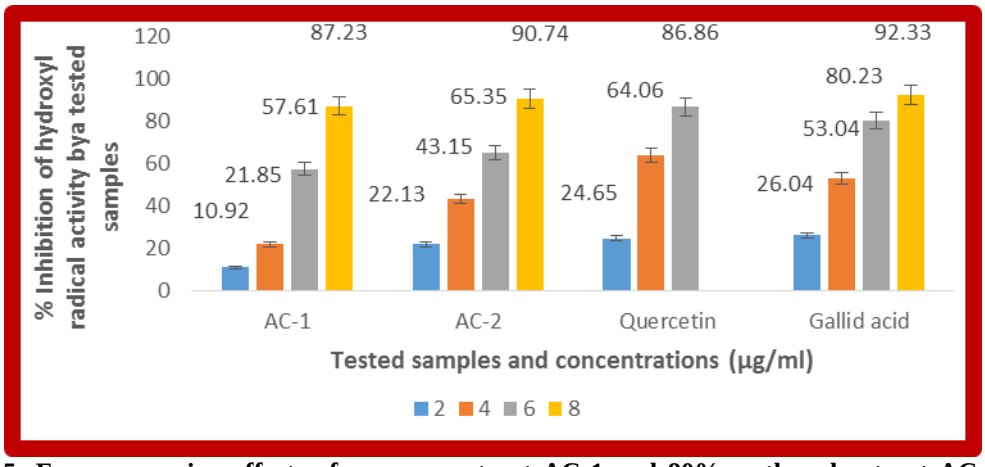

Figure 15: Free scavenging effects of aqueous extract AC-1 and 80% methanol extract AC-2, Quercetin and Gallic acid on hydroxyl ($OH^{\cdot}$)radical activity.

Figure 11 indicated the antioxidant and free scavenging activities of aqueous extract AC-1 and 80% methanol extract AC-2, Quercetin and Gallic acid on hydrogen peroxide radical activity. Results disclosed the effects of aqueous AC-1 and 80% methanol AC-2 extracts showing linhibition percentages from 9.93 to 65.25 for aqueous AC-1 extract low than 50%, from 19.96 to 69.43 for methanol 80 AC-2 extract, from 19.86 to 86.43% for

Quercetin, from 42.12 to 88.74% for Gallic acid towards hydrogen activity when tested at concentrations from 2 to 10 µg/ml The high inhibition percentages were obtained with the highest concentration of 8 µg/ml for Quercetin (86.43%), Galli acid (88.74%°) at at 10 µg/ml for aqueous AC-1 and methanol 80% AC-2 extracts with 65.25 and 69.43% respectively. The most active was Gallic acid an the less active was aqueous extract (Fig.11).

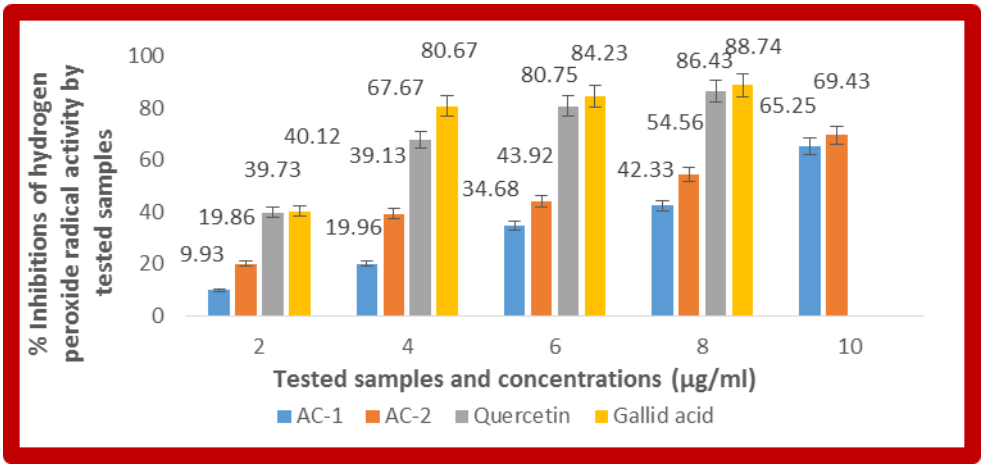

Figure 16: Free scavenging effects of aqueous extract AC-1 and 80% methanol extract AC-2, Quercetin and Gallic acid on hydrogen peroxide activity.

In both evaluated biological activities, it was observed that 80% methanol AC-2 extract exerted high activity compared to aqueous AC-1 extract due to the difference nature of extractive solvent (water versus 80% methanol) and soluble fractions showed weak activity compared to parent aqueous AC-1 extract suggesting that they can react in synergistic manner to restore the high activity of the parent extract (Tables 1 and 2). Ethylacetate AC-1.2 soluble fractions exhibited prominent activities compared to the remaining soluble fractions (Tables 1 and 2). Isolated compound such as Boonein, Echitamie, 6,7 Secoangustilobine and beta-amyrine were not tested for their potential activity since they were in advance considered to be devoid with this activity because their structures did'nt include any phenolic group.

4. CONCLUSION

This study reported for the first time the antispasmodic and antioxidant activities of extracts, fractions and isolated compounds from *Alstonia congensis* root bark on isolated guinea-pig ileum and a large game of reactive oxygen species respectively. Results showed that extracts and fractions exhibited good spasmolytic activity and can particularly support and justify the use of aqueous extract in traditional medicine for the treating of diarrhea. They also showed good and interesting antioxidant activity and can be used for the prevention of the occurring and the management of some degenerative diseases particularly cardiovascular diseases. Future investigations were envisaged for the isolation of active constituents for these evaluated biological activities.

REFERENCES

1. Ahmad I, Khan AU, Chaudhary BA, Jambaz KH, Uzair M, Aktar M, Gilai AH. Antifungal and antispasmodic activities of the extracts of Euphorbia granulate. J Med Plants Res, 2012; 6(1): 19.23.
2. Al-Laith AA, Alkhuzai J, Freije A. Assessment of antioxidant activities of three wild medicinal plants from Bahrain. Arabian J Chem, 2019; 12(8): 2365-2371.
3. Aleem A, Jambaz KH. Dual mechanisms of antimuscarinic and Ca^{2+} antagonistic activities to validate the folkloric uses of Cyperus neveus Retz. as antispasmodic and antidiarrheal. J Ethnopharmacol, 2018; 213: 138-148. doi: 10.1016/j.jep.2017.11.006.
4. Ali N., Alam H, Khan A, Ahmed G, Shah WA, Nabi M, Junaid M. Antispasmodic and antidiarrhoeal activity of the fruit of Rosa moschata (J). BMC Complement Altern Med, 2014. https://bmccomplementalternmed.biomedcentral.com/articles/10.1186/1472-6882-14-485
5. Anonyme. Calcium channel blocker. https://en.wikipedia.org/wiki/Calcium_channel_blocker, 2024.
6. Anuj Y, Rewa K, Ashwani Y, Mishra JP, Seweta S, Shashi P. Antioxidants and its functions in human body-A review. Res Environ Life Sci., 2016; 9(11): 1328-1331.
7. Asifa S, Mueen A, Madiha H, Alageer, Safur RM. Pharmacological evaluation of antispasmodic and brondidilator effects of Sinacia oleraceae L. Am J Phytomed Clin Ther, 2017; 3(2): 1-5.
8. Ayoka TO, Ezema BO, Eze CH, Nnadi CO. Antioxidants for the prevention and treatment of non-Communicable diseases. J Explor Res Pharmacol., 2022; 7(3): 179-189.
9. Brieger K, Shiavau S, Miller FJ, Krause KH. Reactive oxygen species: from health to disease, 2023. DOI: https://doi.org/10.4414/smw.2012.13659

10. Chavan NS, Kardite PB, Despande KG, Kotgire MM. Studies on superoxide anion radical scavenging activity, antioxidant activity, and reducing power of methanolic and aqueous extracts of different medicinal plants. J Pharmacogn Phytochem, 2018; 7(6): 1263-1267.
11. Chaves N, Santiago A, Alías. JC. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. Antioxidants (Basel), 2020; 9(1): 76. doi: 10.3390/antiox9010076
12. Cimanga K R., Nsaka Lumpu, S.., Tshodi Ehata, M.., Kikweta Munduku C.., Kambu Kabangu, O., Apers, S.., Vlietinck, A.J., Pieters, L.. Assessment of antidiabetic activity of the 80% methanol extract of the stem bark of Alstonia congensis Engl. (Apocynaceae) in Wistar rats. World J Pharm Pharm Sci., 2016; 5: 112-134.
13. Cimanga KR, Tshilembi JB, Kikweta MC, Tshodi EM. Spasmolytic and antioxidant activities of aqueous extract and its soluble fractions, and methanol 80% extract of Diodia sarmentosa Swartz (Rubiaceae) leaves. World J Pharm Pharm Sci., 2024; 13(3): 861-883.
14. Cimanga, K., Mukenyi, P.N.K., Kambu, K., Tona, L., Apers, S., Totté, J., Pieters, L. Vlietinck, A.J.. The spasmolytic activity of extracts and some isolated compounds from the leaves of Morinda morindoides (Baker) Milne-Redh. (Rubiaceae). J Ethnopharmacol, 2010; 127: 215-220.
15. de Vargas, F.S., Amleida, P.D.O., de Boleti, A.P., Pereira et al.,. Antioxidant activity and peroxidase inhibition of Amazonian plants extracts traditionally used as anti-inflammatory. Complement Altern Med, 2016; 16: 83. doi: 10.1186/s12906-016-1061-9.
16. Dom NSM, Yahaya N, Adam Z, Rahman NMAN, Hamid M. Antiglycation and Antioxidant Properties of Ficusdeltoidea Varieties. Evid-Based Complement Alternative Med, 2020, 2020: 1-16. https://doi.org/10.1155/2020/6374632.
17. Ehlert FJ, Sawyer GW, Esqueda EE. Contractile role of M2 and M3 muscarinic receptors in gastrointestinal smooth muscle. Life Sci., 1999; 64(6-7): 387-94. doi: 10.1016/s0024-3205(98)00584-0.
18. Forman HT, Zhang H. Targeting oxidative stress in disease: promise and limitation of antioxidant therapy. Nat Rev Drug Discover, 2021; 20: 689-709. https://doi.org/10.1038/141572-021-00233-1.
19. Gilani, AH, Khan A, Ghayur MN. Ca^{2+} antagonist and cholinergic activities explain the medicinal use of olive in gut disorders. Nutrit Res, 2006; 26: 277-283.
20. Gilani AH, Mandukhail SR, Iqbal J, Yasinzai M, Aziz N, Khan A, Rehman N. Antispasmodic and vasodilator activities of Morinda citrifolia root extract are mediated through blockade of voltage dependent calcium channels. Complenent Alternat Med., 2010; 10(2): 1-16., 2023.
21. Guchu BM, Machocho AK, Mwihia SK, Ngugi MP. In Vitro Antioxidant Activities of Methanolic Extracts of Caesalpinia volkensii Harms., Vernonia lasiopus O. Hoffm., and Acacia hockii De Wild. Evid-Based Complement Altern Med, 2020; 2020: 1-10. https://doi.org/10.1155/2020/3586268.
22. Jovanović MS, Milutinović M, Branković S, Tatjana Mihajilov-Krstev T et al., Spasmolytic, Antimicrobial, and Antioxidant Activities of Spray-Dried Extracts of Gentiana asclepiadea L. with In Silico Pharmacokinetic Analysis. Plants, 2024; 13(11): 1445. https://doi.org/10.3390/plants13111445.
23. https://www.google.com/search?q=inhibition+of+muscarinic+receptor&rlz=1C1GCEA_enBE1140BE1140&oq=inhibition+of+muscarinic+receptor&gs_lcrp=egzjahjvbwuybggaeeuyotihcaeqabiabdiicaiqabggb4ycagdeaayfhge0gekmjc3mzdqmgoxnagcclacafefj77b3vkhtv8&sourceid=chrome&ie=UTF-8, 2024.
24. https://rota.prota4u.org/protav8.asp?fr=1&g=pe&p=Alstonia+congensis+Engl.,2023
25. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol., 2011; 48(4): 412-422.
26. Khan M, Sohail, Raja1 NI, Asad MJ Zia-ur-Rehman Mashwani ZuR, Antioxidant and hypoglycemic potential of phytogenic cerium oxide nanoparticles. Sc Rep., 2023; 13: 4514. https://doi.org/10.1038/s41598-023-31498-8.
27. Kozlowska M, Scibisz I, Przybyl JL, Laudy AE, Majewska E et al., Antioxidant and antibacterial activity of exracts from selected plant material. Appl Sci., 2022; 12: 9871. https://doi.org/10.3390/app12199871.
28. Kut K, Cieniek B, Stefaniuk I, Bartosz G, Sadowska-Bartosz I. Modification of the $ABTS^{\bullet}$ Decolorization Method and an Insight into Its Mechanism. Processes, 2022; 10(7): 1288. https://doi.org/10.3390/pr10071288.
29. Labiad MH, Harhar H, Ghanimi A, Tabyaoui M. Phytochemical screening and antioxidant activity of Morocan Thymus stureioides extracts. J Mater Environ Sci, 2017; 8(6): 2132-2139.
30. Lalhminghlui K, Jagetia GC. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth in vitro. Future Sci OA, 2018; 4(2): FSO272-FSO293.
31. Lakshmibai R, Amirtham D. Evaluation of the radical scavenging activity of Mimosa pudIAD Thrns. Asian J Phar Clin Res, 2018; 11(11): 153-156.
32. Lenneke C, Cochemé HA. Redox mechanism: Redox metabolism: ROS as specific molecular regulators of cell signaling and function; Mol Cell, 2021; 81(18): 3691-3707.
33. Lumpu, NS., Kikweta, CM, Tshodi, ME, Mbenza, AP, Kambu, OK, Mbamu, BM, Cos, P, Maes, L, Apers, S, Pieters, L, Cimanga, RK. Antiprotozoal screening and cytotoxicity of extracts and fractions

from the leaves, stem bark and root bark of Alstonia congensis. J Ethnopharmacol, 2013; 148: 724-727.
34. Mamta P, Garry D, Kshipra M, Mausam V, Brar SK. Antioxidants: Biotransformations of waste biomass into high value Biochemicals. Springer Science Businees Media, New-York, 2013. DOI: 10.1007/978-1-4614-8005-1-6.
35. Markrane M, Aziz M, Mekhfi H, Zyyat A, Brouham M, Legsyer A et al.,. Antispasmodic and myorelaxant activity of organic fractions from Origanum majorana L. on intestinal smooth muscle of rodents. J Med Plants, 2018; 23(2): 1-11.
36. Martinez-Pérez EF, Juárez Z, Hernández LR, Bach H. Natural antispasmodic source, Stereochemical configuration and biological activity. BioMed Res Int, 2018; 1-32. https://doi.org/10.1155/2018/3819714.
37. McKeever RG, Pate P, Hamilto RJ. Calcium Channel Blockers. https://www.ncbi.nlm.nih.gov/books/NBK482473/, 2024.
38. Muscolo A, Mariateresa O, Giulio T, Mariateresa R. Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. Int J Mol Sci, 2024; 25(6): 3264. https://doi.org/10.339/ijms25063264.
39. Naz SB, Chaudhray MA, Rehman MS. Dual receptor blocker mechanism attributes smooth muscle relaxant effect of Polypodium vulgare Linn. Bangladesh J Pharmacol, 2016; 11: 414-420.
40. Nisar A, Anzar A, Faizana N, Sadique H. Evaluation of antispasmodic activity of petroleum ether extract of seeds of Apium graveolens Linn (PEESAG) on acetylcholine induced contraction in guinea pigs ileum-An experimental study. Int J Pharm Sci Res, 2015; 6(2): 1397-1399.
41. Okoye NP, Idah OV, Ogundeke TO, Builders M, Amadi K. Evaluation of the antispasmodic potential of leaf extracts of Albizia zygia o isolated rabbit jejunum. J Pharmacocogn Phytochem., 2023; 12(2): 24-28.
42. Pleh, Mahmutović L, Hromić-Jahjefendić A. Evaluation of phytochemical antioxidant levels by hydrogen peroxide scavenging assay Ajla. Bioeng Studies, 2021; 2(1): 1-10.
43. Piqué-Borràs MR, Röhrl J, Künstle G. Herbal Amara extract induces gastric fundus relaxation via inhibition of the M2 muscarinic receptor. Neurogastroenterol Motil., 2025; 37(1): e14924. doi: 10.1111/nmo.14924
44. Rajagopal PL, Sreejith KR, Linsha KT, Premaletha K, Nair MB, Aneeshia S. Antioxidant activity of the leaves of Urea lobata Linn. Int J Res & Rev., 2019; 6(5): 82-84.
45. Rajesh R, Jaivel, Marimuthu A, Okaben H, et al, Antifungal metabolite from Mutingia calabura root against early leaf blight of tomato. J Med Plant Res, 2014; 8: 646-656.
46. Ramachandran CD, Si MS, Rosnah I. Spasmolytic effect of citral and extracts of Cymbopogon citratus on isolated rabbit illeum. J Smooth Muscle Res, 2011; 47(5): 143-156.
47. Rauf A, Akram M, Semwal P, Mujawah AAH et al., Andispasmodic potential of medicinal plants: A comprehensive review.. Oxid Med Cellul Long, 2021: 1-12. https://doi.org/10.1155/2021/4889719.
48. Rauf A, Khalli AA, Awadallah S et al., Reactive oxygen species in biological systems: Pathways associated diseases and potential inhibitors-A review. Food Sci Nutr, 2023; 12(2): 675-693.
49. Richards A, Chaurasia S. Antioxidant Activity and Reactive Oxygen Species (ROS) Scavenging Mechanism of Eriodictyon californium, an Edible Herb of North America. J Chem, 2022; 2022: 1-8. doi.org/10.1155/2022/6980121.
50. Rong-Shan Yan SH, Yang XL, Zhong YM,*, Dao-Qi Zhang DQ, Spontaneous Depolarization-Induced Action Potentials of On-Starburst Amacrine Cells during Cholinergic and Glutamatergic Retinal Waves. Cells, 2020; 9(12): 2574. doi: 10.3390/cells9122574.
51. Saini N, Singh GK, Nagori BP. Antispasmodic potential of Umbelliferae plants: Functional approach. Int J Pharm & Biol Arch, 2014; 5(2): 132-136.
52. Sekendar A, Kazi OF, Aziz AR, Aslam H. Antioxidant and cytotoxic activities of methanol extract of Urena lobata (L.) leaves. The Pharm Inov J, 2013; 2(2): 9-14.
53. Smith, A., Adanlawo, I.G. In vitro and in vivo antioxidant activity of saponin extracted from the root of Garcinia kola (Bitter kola) on alloxan-induced diabetic rats. World J Pharm Pharm Sci., 2014; 3(1): 8-26.
54. Tambuli M, Dey N, Thaku N, Nabaneeta G et al.,. Antispasmodic activity of Musa paradisiaca peel aqueous extrac. World J Pharm Res., 2022; 11(12): 1365-1373.
55. Ulewicz-Magulska B, Wesolowski M. Total phenolic contents and antioxidant potential of herbs used for medical and culinary purpose. Plants Food. Hum Nutr., 2019; 74(1): 61-67.
56. Uwada J, Nakazawa H, Muramatsu I, Masuoka T, azawa T. Role of Muscarinic Acetylcholine Receptors in Intestinal Epithelial Homeostasis: Insights for the Treatment of Inflammatory Bowel Disease. https://www.mdpi.com/1422-0067/24/7/6508#:~:text=Int.%20J.%20Mol.%20Sci.%202023%2C%2024(7)%2C%206508%3B%20https%3A//doi.org/10.3390/ijms24076508
57. Vadivel K, Kumar FG, Babu SM. Ex in vivo spasmolytic activity of aqueous extract of flowers of Muntinginia calabura Linn. on exised rabbit jejunum. Pharm Res, 2017; 9(3): 301-303.
58. Wetchakul P, Chonsut P, Punsawad C, Sineenart Sanpinit S. LC-QTOF-MS Characterization, Antioxidant Activity, and In Vitro Toxicity of Medicinal Plants from the Tri-Than-Thip Remedy. Evid-Based Complement Altern Medi, 2022; 2022: 1-10. ttps://doi.org/10.1155/2022/4477003

59. Wiya C, Saemphet K Saemphet S, Bunecharonen W. In vitro analysis of antispasmodic activity of ethanolic extracts of Uvaria rufa Blume and Anomianthus dukcis (Duanl) J. Sinclair on exised rats's ileum. J Comp Clin Pathol, 2018; 27: 295-299. https://doi0org/10.1007/s00580-0717-2588-y.
60. Yandag SAF, Sotoing GT, Zouakeu KSN EL al.,. Quantification of Bioactive Compounds and Evaluation of the Antioxidant Activity of Carissa edulis Valh (Apocynaceae) Leaves. e Sci World J, 2019; 2019: 1-9. https://doi.org/10.1155/2019/7549620