Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.16, No.2, 2025

GC-MS PROFILE AND *INVITRO* ANTI-DIABETIC, ANTI-INFLAMMATORY, AND ANTIOXIDANT POTENTIALS OF THE AQUEOUS LEAF EXTRACT OF *Chromolaena odorata*

Adeyemo .A.G*[1], Awote O.K[1], Igbalaye J.O[1], Kayode B.D[2], Adeniyi O.A[1], Apete S.K [1], Akorede N. D[1], Saibu G.M[1], Oyekanmi O[1], Lameed A.S[1].

[1]Biochemistry Department, Lagos State University, Ojo, PMB 0001 LASU, Ojo, Lagos, Nigeria
[2] Department of medical science, Eko University of Medicine, and Health science, Lagos Nigeria
*Corresponding Email: adesegun.adeyemo@lasu.edu.ng

ABSTRACT
Chromolaena odorata belongs to the family Asteraceae which is traditionally used as antidiarrheal, astringent, anti-inflammatory, wound healing, anti-hypertensive, and anti-pyretic. The aim of this study was to determine the anti-diabetic, anti-inflammatory, and antioxidant potentials of the aqueous leaf extract *C. odorata*. The phytoconstituents were assayed using GC-MS analysis. The *In-vitro* anti-diabetic activity (alpha amylase inhibitory activity) was assayed, anti-inflammatory activity (albumin denaturation, trypsin inhibitory activity and lipoxygenase activity) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Potential (FRAP), Reducing power and ABTS assays were carried out using standard procedure. The result revealed that the aqueous leaf extract of *Chromolaena odorata* had an anti-diabetic potential by inhibiting alpha amylase activity when compared to the standard (Acarbose). The aqueous leaf extract of *Chromolaena odorata* had a significant ($p<0.05$) increased anti-inflammatory activity by inhibiting albumin denaturation, trypsin activity, and lipoxygenase activity. *C. odorata* aqueous leaf extract had a significantly lowered IC_{50} for ABTS (0.057mg/ml) and DPPH (0.073mg/ml) scavenging activity compared to the standard (Ascorbic acid). In conclusion, the aqueous leaf extract of *C. odorata* exhibited anti-diabetic, anti-inflammatory, and antioxidant activity which could be useful in various fields such as in healthcare, industrial purposes, food.
KEYWORDS: *Chromolaena odorata*, Diabetes, Inflammation, Antioxidant, GCMS
DOI: 10.7176/JNSR/16-2-07
Publication date: November 30th 2025

INTRODUCTION

Since inception, plants have been recognized to contain natural products which serve as food (Liu, 2004) as well as medicine in the event of human infections (Vital and Rivera, 2009). The anti-diabetic, anti-inflammatory, antioxidant, and other effects of any plant depend on the availability of specific phytochemicals. Additionally, they are in charge of the flavors, scents, colors, and, most importantly, the plants' defense mechanisms or resistance to certain infections. (Odutayo *et al*., 2017). There has been quest for search of an alternative phytochemical agents from natural plant origin due to increasing drug resistance by microorganism and the side effects (Paz *et al*., 1995). Microorganisms, particularly bacteria, quickly develop resistance to new drugs when they are first introduced into clinical practice. In other words, an increase in drug resistance has always accompanied the introduction of new chemotherapeutic drugs. (Dhanavade *et al*., 2011). The problem of drug resistance has prompted researchers to turn their attentions to folk medicines as alternative to conventional chemotherapeutic agents following several reports on the medicinal opportunities derived from higher plants (Pimenta *et al*., 2003).

Chromolaena odorata L. (King and Robinson), a member of the Asteraceae family, is one of these plants. Tropical Africa, North America, and South and Southeast Asia all include this weed. Siam weed, Christmas shrub, and common floss flower are some of the common names. (Chakraborty *et al*., 2011). Traditional medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic (Suksamrarn *et al*., 2004). Secondary metabolites with therapeutic value include alkaloids, tannins, flavonoids, and other phenolic compounds found in this plant. The typical effects of plant secondary metabolites include impairments in membrane structure and function, interference with DNA/RNA synthesis, intermediate metabolism, cytoplasmic component coagulation, and interference with appropriate cell communication. (Radulovic *et al*., 2013). Pocket of studies have demonstrated biological activities of plants: phyto-pathogenic activity (Sukanya *et al*., 2009), anthelmintic activity, antiviral activity (Pisutthanan *et al*., 2013) as well as antiprotozoal activity. Ijato and Tedela (Ijato and Tedela, 2015) demonstrated that *C. odorata* extract has inhibitory effect against fungal deteriorating agents of yam tubers. Additionally, some writers showed

that *C. odorata* extract displays bacterial activity and includes several phytochemicals. (Okigbo and Ajalie, 2005).

Naturally, plants possess both primary and secondary metabolites which are important products. The fresh leaf extract of *C. odorata* is used as traditional herbal treatment and medicinal uses in some developing countries. This plant was used for burns, soft tissue wounds and skin infections (Banerjee *et al*., 2017). The anti-diabetic effect of the aqueous leaf extract of *C. odorata* has not been reported. The anti-inflammatory and antibacterial properties of *C. odorata* utilizing the aqueous leaf extract have received less attention. This justifies the current investigation into the anti-diabetic, anti-inflammatory, and antioxidant properties of *Chromolaena odorata's* aqueous leaf extract.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT

Plant was collected from Badagry West Local Council Development Area, Owode Apa, Irosu village and identified at Lagos State University Ojo, Faculty of Science, Department of Botany.

AQUEOUS EXTRACT PREPARATION

80g of the fresh leaves was weighed in 1000ml of deionized water and blended. The aqueous extract of *C. odorata* was heated at 70℃ in a water bath for 2hours. The extract was obtained by filtration using Whatman no. 1 filter paper. Then the filtrates were centrifuged at 5000(rpm) for 5min to have a clear solution (supernatant). These filtrates were stored in the refrigerator for further use.

GC-MS ANALYSIS

GC-MS QP2010 Plus (Shimadzu, Japan) was used in the characterization. The identification of the photochemical in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60m, XTI-5). The initial column temperature was 80ºC for 1min, and then increased linearly at 70ºC min^{-1} to 220ºC, held for 3 min followed by linear increased temperature 10ºC min^{-1} to 290ºC for 10min. The temperature of the injection port was 290ºC and the GC-MS interface was maintained at 290ºC. The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min^{-1}.

IN-VITRO ANTIDIABETIC ACTIVITY

This assay was carried out using a modified procedure of McCue and Shetty (McCue and Shetty, 2004). A total of 250μL of extract (1.25–10mg/mL) was placed in a tube and 250μL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was added. This solution was pre-incubated at 25◦C for 10min, after which 250μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25◦C for 10min. The reaction was terminated by adding 500μL of Dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water and the absorbance was measured at 540nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition: %Inhibition = [Abs control – Abs extracts/Abs control] × 100. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

IN-VITRO ANTI-INFLAMMATORY

ANTI-LIPOXYGENASE ACTIVITY OF THE EXTRACT

Using linoleic acid as substrate and lipoxidase as enzyme, the activity of anti-lipoxidase was studied (Shinde *et al*., 2021). 2M (0.25 ml) borate buffer with pH 9.0 was mixed with 0.25ml (20 000U/mL) lipoxidase enzyme solution and incubated at 25^{O}C for 5 min 1 ml (0.6mM) linoleic acid solution was added to solutions and well mixed. Absorbance was read at 234nm. Indomethacin was used as reference standard. The percentage of inhibition was calculated as follows: Percentage Inhibition = (Control – Test) / Control x 100 Control is the absorbance of solution without extract.

INHIBITION OF ALBUMIN DENATURATION

The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37^0C HCl. The sample extracts were incubated at 37^0C for 20 min and then heated to 51^0C for 20min after cooling the samples the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate (Jayeola *et al*., 2021). Percent inhibition of protein denaturation was calculated as follows: Percentage Inhibition = (Control – Test) / Control x 100 Control is the absorbance of solution without extract.

TRYPSIN INHIBITORY ACTION OF *C. Odorata* AQUEOUS EXTRACT

The reaction mixture (2ml) was containing 0.06mg trypsin, 1ml of 20mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations. The reaction mixture was incubated at 37^0C for 5min and then 1ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20min, 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank (Sakat *et al*., 2010). The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated as follows: Percentage Inhibition = (Control – Test) / Control x 100 Control is the absorbance of solution without extract.

ANTIOXIDANT EVALUATION

DPPH RADICAL SCAVENGING ACTIVITY.

The Antioxidant potential of the synthesized silver nanoparticles was determined based on their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH (24 mg) was dissolved in 100 ml methanol (stock solution). In a test tube, 3 mL DPPH solution was mixed with 100 µL extract solution, different concentrations of the extract solution was used (1–5mg/ml). The absorbance was measured at 517 nm for a period of 30 min. The percentage antioxidant or radical scavenging activity was calculated using the following formula: %Antioxidant activity = [(Ac − As)/Ac] × 100 where, Ac and As are the absorbance of control and sample, respectively. The control contained 100 µL distilled water in place of the extract solution (Shah *et al*., 2013).

FERRIC REDUCING ANTIOXIDANT POTENTIAL (FRAP)

FRAP reagent was prepared by mixing in 25 mL acetate buffer (30 mM; pH 3.6), 2.5 mL TPTZ solution (10 mM) and 2.5 mL ferric chloride solution (20 mM). The mixture was incubated for 15 min at 37 °C before use. Ascorbic acid (vitamin C) was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 50 mg/L to 500 mg/L in water. To 2.85 mL FRAP reagent in a test tube, 150 µL extract solution was added, different concentrations of extract solution (1- 5mg/ml) were used. The mixture was incubated for 30 min in the dark, and its absorbance was measured at 593 nm. The blank contained an equal volume of methanol instead of the plant sample. The results were reported as µg of ascorbic acid equivalents (AAE) per mL (Huang *et al*; 2005).

HYDROGEN PEROXIDE SCAVENGING CAPACITY

The ability of the *C.odorata* extract to scavenge hydrogen peroxide was determined according to the method by (Keser *et al*., 2012). A solution of hydrogen peroxide (40 mm) was prepared in phosphate buffer (pH 7.4). Extracts (1-5mg/ml) were added to a hydrogen peroxide solution (0.6 ml, 40mm). The absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of *C.odorata* extract and standard (Ascorbic acid) compounds were calculated: Percentage Inhibition = (Control – Test) / Control x 100

Where **AC** is the absorbance of the control and **AS** is the absorbance in the presence of the sample of *C.odorata* extract or standards.

ABTS$^{\bullet+}$ DECOLORIZATION ASSAY

The working solution of ABTS•+ radical was made by reacting ABTS (9.5 mL, 7 mM) with potassium persulfate (245 µL, 100 mM), and raising the volume to 10 mL with distilled water. The solution was kept in the dark at room temperature for 18 h, and then diluted with potassium phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 (±0.02) at 734 nm (Bursal and Gülçin, 2011). 10 µL of distilled water was placed in a test tube and mixed thoroughly with 2.99 mL ABTS radical working solution, different concentrations of extract solutions (0.2-1mg/ml) were used. Absorbance of the resulting clear mixture was recorded at 734 nm. The percent antioxidant activity of the sample was determined using the following formula:

%Antioxidant activity = [(Ac − As)/Ac] × 100 ; where Ac and As are the absorbance of the control and sample, respectively. The control was prepared by adding 10 µL of distilled water solution in place of the sample.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of mean (SEM) and values were considered significant when $p<0.05$. Statistical comparisons between all groups were performed by using Graph pad prism.

RESULTS

Figure 1 shows the GC–MS analysis of *C. odorata* revealed the presence of various phytochemicals with different retention time and relative concentration.

GC-MS ANALYSIS

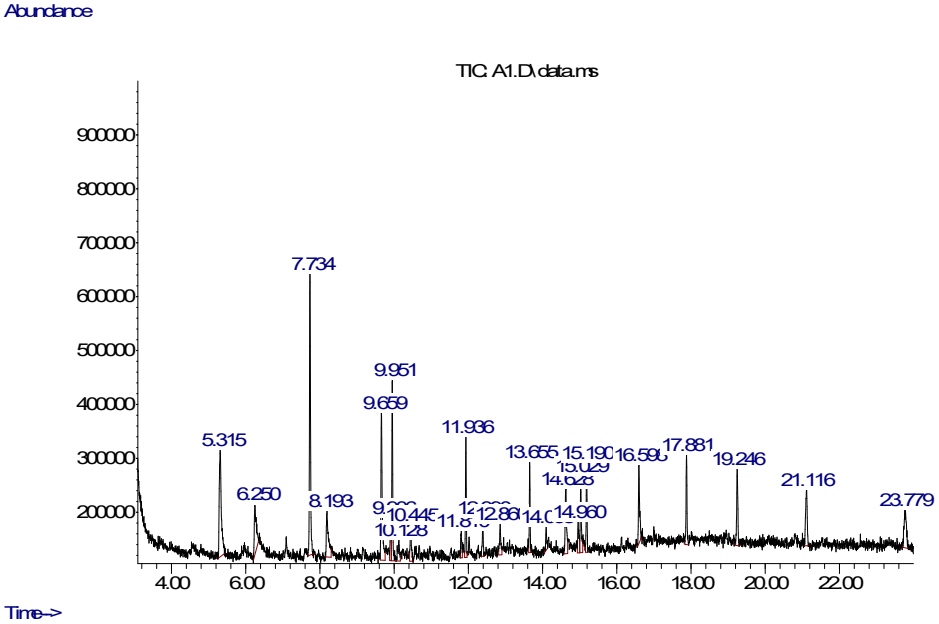

Fig: 1: Gas Chromatogram of aqueous leaf extract of *Chromolaena odorata* showing the peak and abundance of phytoconstituents present in the extract.

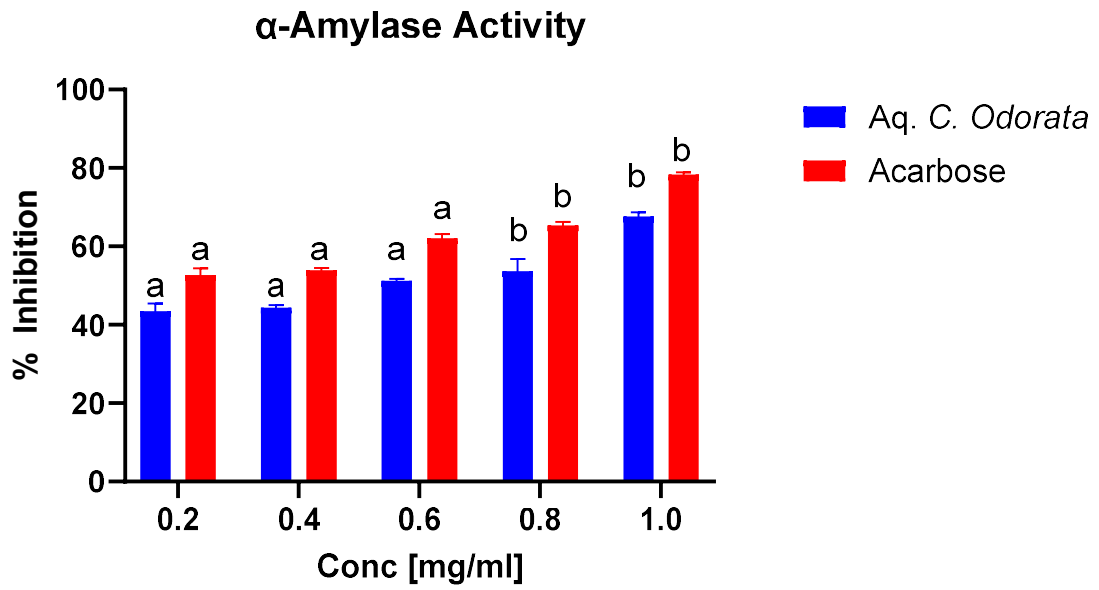

Fig 2: Alpha amylase % inhibitory activity of *Chromolaena odorata* extract compared with standard (Acarbose). Each bar represents mean ± SD. Each alphabet represents significant difference ($p<0.05$) between the extract and standard.

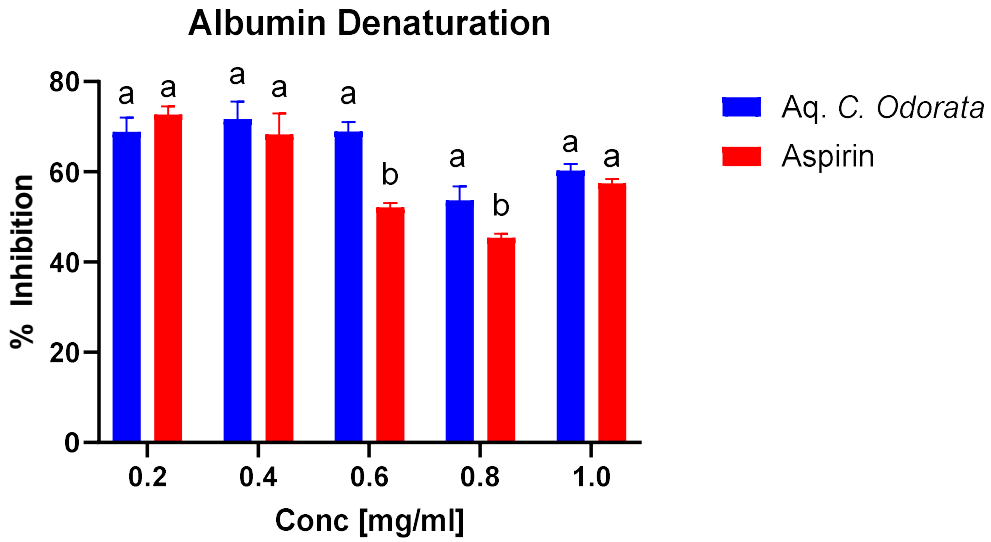

Fig 3: Albumin denaturation inhibitory activity of *Chromolaena odorata* extract compared with standard (Aspirin). Each bar represents mean ± SD. Each alphabet represents significant difference ($p<0.05$) between the extract and standard.

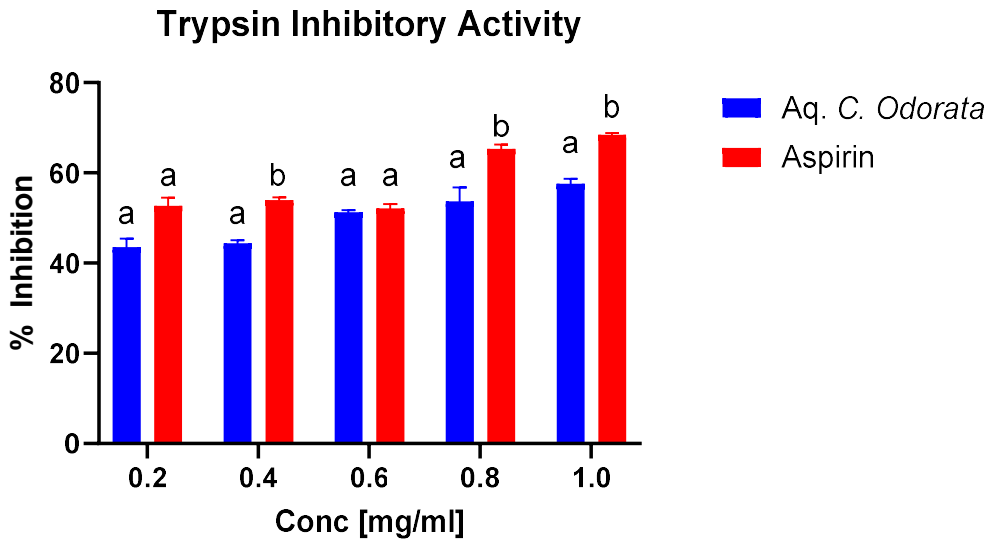

Fig 4: Trypsin inhibitory activity of *Chromolaena odorata* extract compared with the standard (Aspirin). Each bar represents mean ± SD. Each alphabets represents significant difference ($p<0.05$) between the extract and the standard.

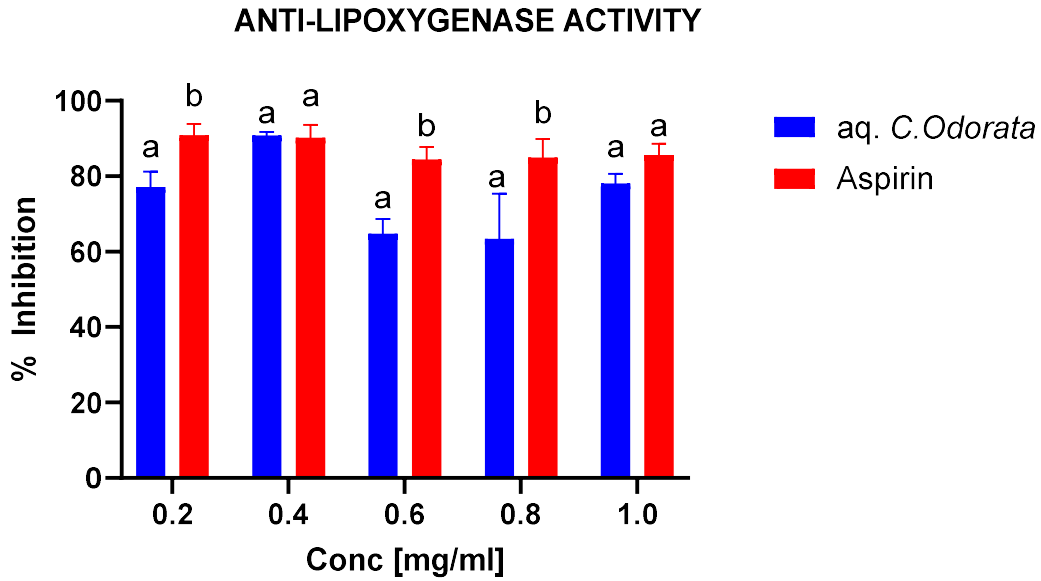

Fig. 5: Anti-lipoxygenase inhibitory activity of *Chromolaena odorata* extract compared with standard (Aspirin). Each bar represents mean ± SD. Each alphabet represents significant difference ($p<0.05$) between the extract and the standard.

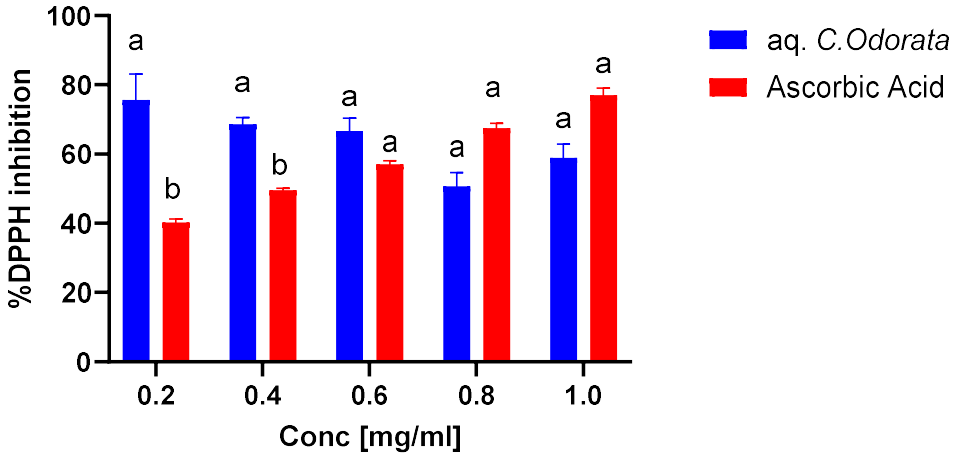

Fig 6: Percentage DPPH inhibition of *Chromolaena odorata* extract compared with the standard (Ascorbic acid). Each bar represents mean ± SD. Each alphabets represents significant difference ($p<0.05$) between the extract and the standard.

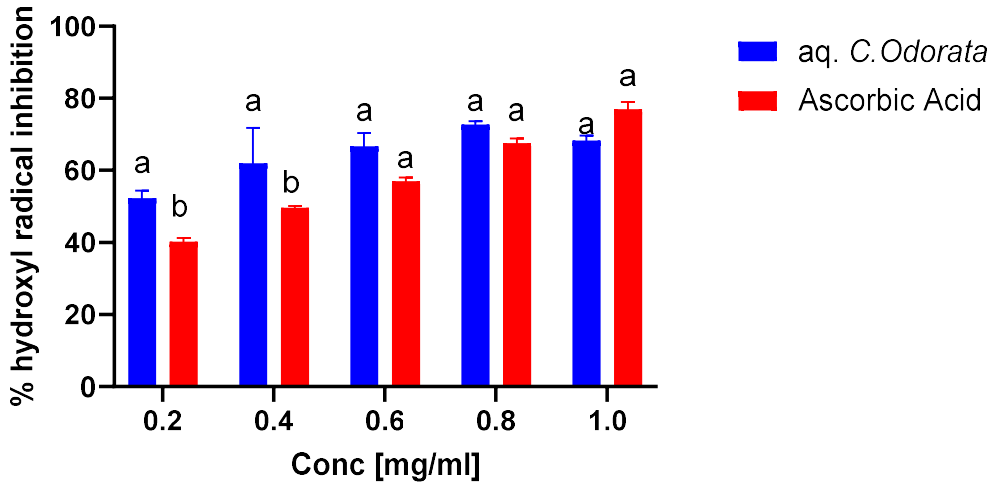

Fig 7: Percentage Hydroxyl radical inhibition of *C. odorata* extract compared with the standard (Ascorbic acid). Each bar represents mean ± SD. Each alphabets represents significant difference ($p<0.05$) between the extract and the standard.

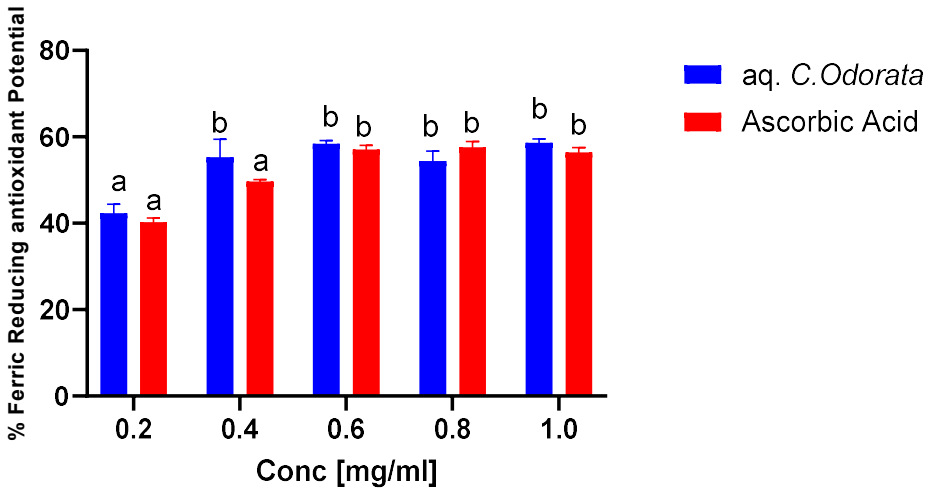

Fig 8: Percentage Ferric Reducing Antioxidant Potential of *C. odorata* extract compared with the standard (Ascorbic acid). Each bar represents mean ± SD. Each alphabets represents significant difference ($p<0.05$) between the extract and the standard.

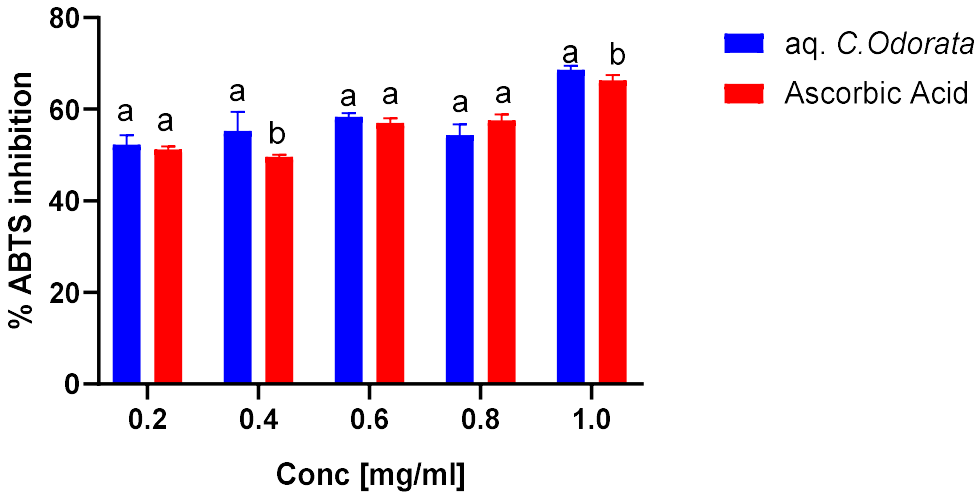

Fig 9: Percentage ABTS Inhibition of *C. odorata* extract compared with the standard (Ascorbic acid). Each bar represents mean ± SD. Each alphabets represents significant difference ($p<0.05$) between the extract and the standard.

Table 1: Antioxidant IC_{50} value of aqueous extracts of *C. odorata*

Extracts (mg/ml)	ABTS Scavenging Activity (mg/ml)	Ferric reducing Antioxidant Power (mg/ml)	Hydrogen peroxide scavenging activity (%)	DPPH Scavenging Activity (mg/ml)
Aqueous C.Odorata	**0.057 ± 0.002[a]**	**0.058 ± 0.001[a]**	**58 ± 0.004[a]**	**0.073 ± 0.003[a]**
Ascorbic Acid	**0.131 ± 0.010[b]**	**0.056 ± 0.008[a]**	**63 ± 0.001[b]**	**0.161±0.010[b]**

The values are expressed as mean ± SEM of triplicate determinations. Means down vertical column not sharing a common superscript are significantly different ($p < 0.05$) from each other. Ascorbic acid is the standard antioxidant agent.

DISCUSSION

The quantitative analysis of phytochemical components conducted by (Melinda *et al*., 2010) showed that *C. odorata* had a high total phenol component. In addition, (Omokhua *et al*., 2016) reported that based on the results of qualitative analysis of phytochemical components *in C. odorata* plants contained phenols, flavonoids, saponins, and tannins in both biotypes, namely Asian/West African Biotype and Southern African Biotype. In the present study, research has been carried out to evaluate the preliminary phytoconstituents investigation and the potential of the aqueous leaf extract of *Chromolaena odorata* in inhibiting alpha-amylase. The present finding of Phytoconstituents screening of the plant extract confirmed the presence of several compounds in Fig. 1 which could be responsible for the versatile medicinal properties of this plant.

The GC–MS analysis of *C. odorata* revealed the presence of various phytochemicals with different retention time and relative concentration. A mass spectrometer analyzes fragments with different m/z ratios, which were identified from the fingerprint data library. The pharmacological activities of identified molecules from GC–MS analysis was searched for reports in published literature. A subset of phytochemicals with known relevant (i.e., antioxidant, anti-infammatory or antidiabetic) pharmacological properties has been presented in this research. The major constituents (based on area) in *C. odorata* extract were Cyclooctene, 4-methylene-6-(1-prop enylidene), 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetra siloxane, Octasiloxane, 2,4-Hexadiyne, Benzenesulfonic acid. It should be noted that plant extracts could have several metabolites and only a fraction of those could be active/efective in the *in vitro* assay depending on whether they are bioactive or bioavailable (Khan *et al*. 2017). Though all referred phytochemicals in the present study suggest one or more molecules could be linked to the observed inhibition of α-amylase (anti-diabetic), antioxidant and anti-inflammatory activity of *C. odorata* aqueous leaf extract. The vast majority of the phytoconstituents have been accounted from these plants, however, pharmacological actions have not been specified. Since the nature of the phytochemical responsible for the inhibitory activity has not yet been ascertained, the principle active molecules(s) need to be isolated and characterized through in vitro and in vivo studies.

Postprandial high blood glucose, related to type 2 diabetes, is one of the earliest methods of glucose homeostasis (Awote *et al*., 2021). The usual pharmacological approach to this condition has been the use of therapeutic agents such as medicinal plants consisting of important potential phytochemicals sources used for the treatment and management of Type 2 Diabetes Mellitus. Several reports showed that this therapeutic approach can decrease postprandial hyperglycaemia by inhibiting carbohydrate digesting enzymes (e.g α-amylase) resulting in a delay of carbohydrate digestion to absorbable monosaccharides (Kashtoh et al., 2023). In this research work, α-amylase inhibitory activities of aqueous extracts of *C. Odorata* was evaluated to clarify its traditional claim and use for diabetes treatment and management. Our obtained result of *in vitro* analysis of the activity of α-amylase inhibition, slightly found in a dose-dependent manner confirms that the high amount of bioactive compounds (flavonoids, phenolic acids, and alkaloids) in the plant extracts at different concentrations may be responsible for the inhibitory activity of α-amylase (Kifle and Enyew, 2020). Several phytochemical analyses have reported that extracts rich in polyphenolic components such as flavonoids have an α-amylase inhibitory activity that depends on phenolic profile (Rana et al., 2019). More, so, the aqueous leaf extracts of *C. Odorata* showed α-amylase

inhibitory properties at all the concentrations, suggesting that the plant may be useful in the management of post-prandial glucose in diabetes mellitus.

The present finding reveals that *C. odorata* efficiently inhibits alpha amylase activity (Fig. 2). As the concentration was increasing, there was a significant increase in the inhibition of alpha amylase activity compared with the standard (Acarbose). The anti-diabetic action of *C. odorata* could be attributed to the phytochemicals present in the extract which are acting as to inhibit alpha amylase activity.From the calculated percentage of inhibition values it becomes evident that the aqueous leaf extract of *C. odorata* can serve as an anti-diabetic agent.

Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins (Chandra *et al*., 2012). Agents that can prevent protein denaturation, therefore, would be worthwhile for anti-inflammatory drug development. Hence, in the present study, the *In-vitro* anti-inflammatory effect of the aqueous leaf extract of *Chromolaena odorata* was evaluated against denaturation of albumin. Aspirin (at the concentration of 0.8g/mL) was used as reference drug which significantly exhibited higher inhibition of albumin denaturation. The increase in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by the aqueous leaf extract of *Chromolaena odorata* and reference drug Aspirin (Onoja *et al*., 2016). From the calculated percentage of inhibition values it becomes evident that the aqueous leaf extract of *Chromolaena odorata* can serve as an anti-inflammatory agent. Polyphenols are well known natural products known to possess several notable biological properties (Tsao, 2010). In the present study, the *in-vitro* anti-inflammatory activity of *Chromolaena odorata* can be attributed to its polyphenols, alkaloids, flavonoids and saponin content. The effect may be due to the synergistic effect rather than single constituent. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (6.2-6.5) (Chatterjee *et al*., 2012). Therefore, from the results of the present study it can be concluded that *Chromolaena odorata* leaf extract exhibited marked *in-vitro* anti-inflammatory effect against the denaturation of albumin. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions.

The aqueous leaf extract of *C. odorata* exhibited a significant inhibition of trypsin activity compared with the standard in Fig 4.4. The inhibition is as a result of the phytochemicals present in the aqueous leaf extract of *C. odorata. Chromolaena odorata* has been shown to contain the flavonoids quercetin, isosakuranetin, and sakuranetin (Baldoqui *et al*., 2019). Quercetin and other flavonoids possess anti-inflammatory activity (Gonzalez-Galarza *et al*., 2020). It is not clear however if the activities in this study were due to flavonoids alone or other bioactive components in the plant. Therefore, the aqueous leaf extract of *C. odorata* displayed marked anti-inflammatory activity against trypsin inhibition.

Lipoxygenase is responsible for the synthesis of inflammatory lipid mediators, such as leukotrienes, lipoxins, hepoxilins, and other hydroxylated fatty acid derivatives (Kuhn and McPartland, 2017). The ability to inhibit lipoxygenase activity by the aqueous leaf extract of *C. odorata* was significant compared with the standard. It was revealed that the inhibition of lipoxygenase activity by the aqueous leaf extract of *C. odorata.* from the calculated percentage of inhibition values significantly had the same activity as the standard (Aspirin) employed in this study. Previous reports showed that the aqueous leaf extract of *C. odorata* significantly reduced the carrageenan-induced oedema, cotton pellet granuloma and formalin-induced oedema in rat models. Therefore, *Chromolaena odorata* can serve as an anti-inflammatory agent by inhibiting the activity of lipoxygenase.

Antioxidants can be classified into two broad categories – primary (chain breaking) antioxidants and secondary (preventive) antioxidants – depending on their mode of action (Koh *et al*., 2019). Primary antioxidants, also known as class I antioxidants, typically act as acceptors of free radicals, thereby inhibiting the initiation step or interfering with the propagation step of autoxidation (Daramola and Adegoke, 2011). Thus, primary antioxidants must be able to donate a hydrogen atom to the free radical (Berdahl *et al*., 2010). On the other hand, secondary antioxidants, also known as class II antioxidants, slow down the rate of oxidation reactions through many different mechanisms (Mishra and Bisht, 2011). For instance, secondary antioxidants may provide H to a primary antioxidant, function as oxygen scavengers, or decompose hydrogen peroxide into non-radical species (Wanasundara and Shahidi, 2005). In essence, primary antioxidants convert free radicals into stable molecules while secondary antioxidants do not. Instead, secondary antioxidants often enhance the antioxidant activities of primary antioxidants (Mondal, 2017).

DPPH is a stable radical; the model of scavenging DPPH radical is a widely used method to evaluate the primary antioxidant potential in a relatively short time compared with other methods. In the presence of hydrogen donating antioxidant, the stable DPPH radical is converted into a non-radical component (DPPH-H), and due to this reaction that the color of the DPPH solution is converted from purple to yellow (Wang *et al*., 2015). The *C. Odorata* aqueous extract had an IC_{50} of 0.058 ± 0.0052 mg/ml against DPPH coinciding with the result of (Palanisamy *et al*., 2017a) having an IC_{50} of 0.089 mg/ml, due to the lower IC_{50} of *C. Odorata* aqueous extract it has more potency than ascorbic acid. It was evident that the *C. Odorata* aqueous extract did show strong proton-donating ability and could serve as free-radical inhibitors or scavengers, acting possibly as primary antioxidants (Wang *et al*., 2010a). Ferric Reducing Antioxidant Power assay is used to analyze if a natural compound can reduce iron. In this study *C. Odorata* aqueous extract had the same reducing strength significantly with the standard (Ascorbic acid), having an IC_{50} of 0.058±0.016 mg/mL. This result is similar to (Palanisamy *et al*., 2017). Hydrogen peroxide itself is not very reactive, but sometimes it is toxic to cells because it may give rise to hydroxyl radicals in the cells (Palanisamy *et al*., 2017b). Hence, compounds with good hydrogen peroxide scavenging ability are considered physiologically important. The *C. Odorata* aqueous extract sample exhibited the most potent hydrogen peroxide scavenging activity of about 90% in contrast to the standard (ascorbic acid) which is about 50%.

CONCLUSION

In conclusion, the aqueous leaf extract of *C. odorata* plant showed significant anti-diabetic, anti-inflammatory, and antioxidant activities. Further the compound isolation, purification and characterization which is responsible for inhibitory activity, has to be done for the usage of anti-diabetic, anti-inflammatory, and antioxidant agent.

RECOMMENDATION

Further need to determine the *in-vivo* anti-diabetic, anti-inflammatory, and antimicrobial potentials of the aqueous leaf extract of *C. odorata* and the mechanism of action of the phytochemicals responsible for the inhibitory activity.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCE

AGABA, T., FAWOLE, B. & CLAUDIUS-COLE, A. 2015. Screening of siam weed (Chromolaena odorata) and African custard apple (Annona senegalensis) for nematicidal activity. *Journal of Biology, Agriculture and Healthcare,* 5**,** 50-56.

AHMED, K. B. A., SUBRAMANIAN, S., SIVASUBRAMANIAN, A., VEERAPPAN, G. & VEERAPPAN, A. 2014. Preparation of gold nanoparticles using Salicornia brachiata plant extract and evaluation of catalytic and antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 130**,** 54-58.

BALDOQUI, D. C., MENIQUETI, A. B., RAMOS, A. V. G., SARRAGIOTTO, M. H. & CARMO, M. R. B. D. 2019. Chemotaxonomic implications of methoxy flavonoids in ageratina and chromolaena. *Biodiversity and chemotaxonomy.* Springer.

BANERJEE, J., SINGH, R., VIJAYARAGHAVAN, R., MACFARLANE, D., PATTI, A. F. & ARORA, A. 2017. Bioactives from fruit processing wastes: Green approaches to valuable chemicals. *Food chemistry,* 225**,** 10-22.

BOURDEV, L., MAJI, S., BROX, T. & MALIK, J. Detecting people using mutually consistent poselet activations. European conference on computer vision, 2010. Springer, 168-181.

CHAKRABORTY, A. K., RAMBHADE, S. & PATIL, U. K. 2011. Chromolaena odorata (L.): An overview. *J Pharm Res,* 4**,** 573-576.

CHANDRA, S., CHATTERJEE, P., DEY, P. & BHATTACHARYA, S. 2012. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pacific Journal of Tropical Biomedicine,* 2**,** S178-S180.

CHATTERJEE, P., CHANDRA, S., DEY, P. & BHATTACHARYA, S. 2012. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative in vitro study. *Journal of advanced pharmaceutical technology & research,* 3**,** 136.

DHANAVADE, M. J., JALKUTE, C. B., GHOSH, J. S. & SONAWANE, K. D. 2011. Study antimicrobial activity of lemon (Citrus lemon L.) peel extract. *British Journal of pharmacology and Toxicology,* 2**,** 119-122.

GONZALEZ-GALARZA, F. F., MCCABE, A., SANTOS, E. J. M. D., JONES, J., TAKESHITA, L., ORTEGA-RIVERA, N. D., CID-PAVON, G. M. D., RAMSBOTTOM, K., GHATTAORAYA, G. & ALFIREVIC, A. 2020. Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic acids research,* 48**,** D783-D788.

KASHTOH, H. AND BAEK, K.H., 2023. NEW INSIGHTS INTO THE LATEST ADVANCEMENT IN A-AMYLASE INHIBITORS OF PLANT ORIGIN WITH ANTI-DIABETIC EFFECTS. *PLANTS*, *12*(16), P.2944.

IJATO, J. & TEDELA, P. 2015. Phytotoxic potentials of cold and hot aqueous extracts of Chromolaena odorata against fungal deteriorating agents of yam tubers (Dioscorea rotundata, Poir) after harvest. *American Journal of Experimental Agriculture,* 5**,** 262.

JAYEOLA, D. K., BELLO, T. O., OYEDEPO, V. O. & FAMUREWA, O. C. 2021. The efficacy of oral naproxen and cervical lidocaine spray on perceived pain during hysterosalpingography. *Nigerian Postgraduate Medical Journal,* 28**,** 193.

KARIMI, E., JAAFAR, H. Z. & AHMAD, S. 2011. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of Labisa pumila Benth. *Molecules,* 16**,** 4438-4450.

KIFLE, Z.D. AND ENYEW, E.F., 2020. Evaluation of in vivo antidiabetic, in vitro α-amylase inhibitory, and in vitro antioxidant activity of leaves crude extract and solvent fractions of Bersama abyssinica fresen (melianthaceae). *Journal of Evidence-Based Integrative Medicine*, *25*, p.2515690X20935827.

KUHN, M. H. & MCPARTLAND, T. S. 2017. An empirical investigation of self-attitudes. *Sociological Methods.* Routledge.

LIU, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition,* 134**,** 3479S-3485S.

MCCUE, P. P. & SHETTY, K. 2004. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition,* 13.

MELINDA, K. P., RATHINAM, X., MARIMUTHU, K., DIWAKAR, A., RAMANATHAN, S., KATHIRESAN, S. & SUBRAMANIAM, S. 2010. A comparative study on the antioxidant activity of methanolic leaf extracts of Ficus religiosa L, Chromolaena odorata (L.) King & Rabinson, Cynodon dactylon (L.) Pers. and Tridax procumbens L. *Asian Pacific Journal of Tropical Medicine,* 3**,** 348-350.

MICROBIOLOGY, E. B. T. E. S. O. C., DISEASES, I., INFECTION, B. T. I. S. O. C. F., CANCER, HABIB, G., HOEN, B., TORNOS, P., THUNY, F., PRENDERGAST, B., VILACOSTA, I., MOREILLON, P., DE JESUS ANTUNES, M. & THILEN, U. 2009. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009) The Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). *European heart journal,* 30**,** 2369-2413.

NACZK, M. & SHAHIDI, F. 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of pharmaceutical and biomedical analysis,* 41**,** 1523-1542.

ODUTAYO, F., EZEAMAGU, C., KABIAWU, T., AINA, D. & MENSAH-AGYEI, G. 2017. Phytochemical screening and antimicrobial activity of Chromolaena odorata leaf extract against selected microorganisms. *Journal of Advances in Medical and Pharmaceutical Sciences,* 13**,** 1-9.

OKIGBO, R. & AJALIE, A. 2005. Inhibition of some human pathogens with the tropical plant extracts Chromolineena odorata and Citrus aurantifolia and some antibiotics. *Int. J. Mol. Med. Adu Sci,* 1**,** 34-40.

OMOKHUA, A. G., MCGAW, L. J., FINNIE, J. F. & VAN STADEN, J. 2016. Chromolaena odorata (L.) RM King & H. Rob.(Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. *Journal of ethnopharmacology,* 183**,** 112-122.

ONOJA, S. O., NWEZE, E. C., EZEJA, M. I., OMEH, Y. N. & OBI, C. O. 2016. Evaluation of the antioxidant and analgesic activities of hydromethanolic extract of Chromolaena odorata leaf. *British Journal of Pharmaceutical Research,* 10**,** 1.

PAZ, E. A., CERDEIRAS, M., FERNANDEZ, J., FERREIRA, F., MOYNA, P., SOUBES, M., VAZQUEZ, A., VERO, S. & ZUNINO, L. 1995. Screening of Uruguayan medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology,* 45**,** 67-70.

PIMENTA, L. S., PINTO, G., TAKAHASHI, J. A., E SILVA, L. & BOAVENTURA, M. A. 2003. Biological screening of annonaceous Brazilian medicinal plants using Artemia salina (Brine shrimp test). *Phytomedicine,* 10**,** 209-212.

PISUTTHANAN, S., PLIANBANGCHANG, P., PISUTTHANAN, N., RUANRUAY, S. & MUANRIT, O. 2013. Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. *Naresuan University Journal: Science and Technology (NUJST),* 12**,** 13-18.

RADULOVIC, N., BLAGOJEVIC, P., STOJANOVIC-RADIC, Z. & STOJANOVIC, N. 2013. Antimicrobial plant metabolites: structural diversity and mechanism of action. *Current medicinal chemistry,* 20**,** 932-952.

RANA, Z.H., ALAM, M.K. AND AKHTARUZZAMAN, M., 2019. Nutritional composition, total phenolic content, antioxidant and α-amylase inhibitory activities of different fractions of selected wild edible plants. *Antioxidants*, *8*(7), p.203.

SAKAT, S., JUVEKAR, A. R. & GAMBHIRE, M. N. 2010. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. *Int J Pharm Pharm Sci,* 2**,** 146-155.

SALSABILA, A., HARFIANI, E. & NUGRAHA, Y. The effectivities of anti-diabetic of Chromolaena odorata L. in lowering blood sugar level: A systematic review. IOP Conference Series: Earth and Environmental Science, 2021. IOP Publishing, 012092.

SHINDE, V., BHIKHA, S., HOOSAIN, Z., ARCHARY, M., BHORAT, Q., FAIRLIE, L., LALLOO, U., MASILELA, M. S., MOODLEY, D. & HANLEY, S. 2021. Efficacy of NVX-CoV2373 Covid-19 vaccine against the B. 1.351 variant. *New England Journal of Medicine,* 384**,** 1899-1909.

SUKANYA, S., SUDISHA, J., HARIPRASAD, P., NIRANJANA, S., PRAKASH, H. & FATHIMA, S. 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African journal of biotechnology,* 8.

SUKSAMRARN, A., CHOTIPONG, A., SUAVANSRI, T., BOONGIRD, S., TIMSUKSAI, P., VIMUTTIPONG, S. & CHUAYNUGUL, A. 2004. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers ofChromolaena odorata. *Archives of Pharmacal Research,* 27**,** 507-511.

THOPHON, S. H. S., WARANUSANTIGUL, P., KANGWANRANGSAN, N. & KRAJANGSANG, S. 2016. Antimicrobial activity of Chromolaena odorata extracts against bacterial human skin infections. *Modern Applied Science,* 10.

TIWARI, G., WANG, S., TANG, J. & BIRLA, S. 2011. Analysis of radio frequency (RF) power distribution in dry food materials. *Journal of Food Engineering,* 104**,** 548-556.

TSAO, R. 2010. Chemistry and biochemistry of dietary polyphenols. *Nutrients,* 2**,** 1231-1246.

VITAL, P. G. & RIVERA, W. L. 2009. Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. Extracts. *Journal of medicinal plants Research,* 3**,** 511-518.