

PHYSICOCHEMICAL , MICROBIOLOGICAL AND ANTIOXIDANT ACTIVITY OF THE LEAF EXTRACTS OF FERMENTED *ARTEMISIA ANNUA* VAR. *CHIKNENSIS*. (CBGE/CHNA/09/LTNGS/G) AND *VERNONIA AMYGDALINA* DEL.

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ABSTRACT

This study investigated the physicochemical , microbiological and invitro antioxidant properties of fermented leaf extracts of *Artemisia annua* and *Vernonia amygdalina*. It showed a significant decrease in specific gravity ($P < 0.05$) and very significant decrease ($P < 0.05, 0.01, 0.001$ and specific gravity and pH respectively. There was a significant difference ($P < 0.05$) and very significantly different ($P < 0.05, 0.01, 0.001$) in temperature, titratable acidity and viscosity respectively. there was no significant difference ($P > 0.05$) in alcohol content. The invitro antioxidant property indicated high radical scavenging and reducing power with HRSA% showing highest activity in both extracts. The colony Forming Units of *Lactobacillus* sp and *Saccharomyces cerevisiae* used in fermenting the extracts showed no significant difference ($P > 0.05$). in all dilutons. This study contributes in understanding of quality, consistency and potency of these fermented extracts highlighting their applications in food, pharmaceutical and cosmetic industries.

1. INTRODUCTION

1.0 BACKGROUND

Fermented plant extracts (FPEs) are common food types in Asian countries. FPEs are fermented by numerous microbes including *S. cerevisiae*, molds, *Lactobacillus* sp among others (Blandino *et al.*, 2003) which expedite the transformation of complex sugars into alcohols, proteins into peptides and amino acids while oils and fats are converted into fatty acids and glycerol. This accelerates, bio-digestibility, bio-accessibility, bio-availability and bio absorption of the biochemical compounds for eradication of hyperglycemia and other health challenges (Feng, 2017; Pe-nas *et al.*, 2017). Fermentation allows modifications to occur which results in improved texture, quality, taste, colour, smell, longer shelf life and healthful value (Nkhata *et al.*, 2018; Ross *et al.*, 2018).

Artemisia annua also called qinghao in Chinese (meaning 'blue-green' artemisia), sweet wormwood in English . The plant is native to temperate Asia, but adapted globally, and belongs to the family of the Asteraceae (EI-Askary *et al.*, 2020). The plant is large, weedy, shrubby and grows to a height above 2m, having a ribbed single-stem with alternate branches.

Vernonia amygdalina is a bitter, shrubby plant which grows to a height of about five (5) m high. The various organs on the plant possess several activities such antioxidant, anti-diabetic, anti-inflammatory and so on (Alara *et al.*, 2017). Physicochemical properties are often used in the improvement of new products for domestic, industrial and medical applications. The properties comprise of alcohol contents, pH, viscosity, density, antioxidant capacity, titratable acidity ,conductivity and total solids. Johnson *et al.* (2023) reported increased bioactive components, nutritional and sensory parameters in fermented plant extract compared to the unfermented ones ($P < 0.05$) Ifesan *et al.* (2014) reported pH and temperature increase within ranges of 6.8 -3.78 and 27 – 34°C respectively and 0.014-0.147 lactic acid in the fermentation of green leafy vegetables. Bayoi and Francois -Xavier (2023) reported an alcoholic content of 0-9.5% and 0-6.8% (v/v) after fermentation of Sorghum with decrease in pH from 3.2-2.4 and 3.11-2.41. This reduction in pH may be caused by the production of Various organic acids in the course of the lactic acid fermentation (Giraffa *et al.* 2010). During fermentation of the *A. annua*, pH, acidity and in viable count increased have been reported (On *et al.* , 2023).

Pharmacological effects like Antioxidant activity in *A. annua* have been documented (Lang *et al.*, 2019; Axelle *et al.*, 2020). The mechanism behind enhanced antioxidant activity with reduction in level of antioxidant bioactive components such as polyphenolics and flavonoids in the course of the fermentation is not well understood. Possible

reasons may be the biodegradation of these phytochemicals during fermentation or biosynthesis of some unidentified ones occurred or even that the *Lactobacillus* utilized them as energy sources. Lee *et al.* (2019) described how the fermentation of *A. annua* using *Lactobacillus plantarum* and testing certain parameters such as phytochemical components and antioxidant activity on the antioxidant activity, DPPH free radical scavenging activity for the fermented extract was enhanced above the non-fermented extract. However, on the superoxide radical reduction assay both fermented and non-fermented extracts were enhanced.

The aqueous Leaf extracts of *V. amygdalina* have demonstrated a substantial decrease in the amount of malondialdehyde in oxidative stressed streptozotocin-induced diabetic rats. These extracts were reported to scavenge 75-99.3% and 96.2-100% of DPPH radicals and ABTS radicals respectively.

2. MATERIALS AND METHODS

2.0 Data Collection Techniques

2.1 Preparation of Extracts: Four hundred grams (400g) of powdered (dried) leaves each plant was put in a conical flask containing 2000ml of sterile distilled water. The flask was heated with a Bunsen flame for few minutes and was allowed to cool to room temperature. It was aseptically filtered using Whatman filter paper (No1) to separate the residue from the filtrate.

2.2 Preparation of plant extracts: The extraction of powdered sample of aqueous and methanolic leaf of *A. annua* and *V. amygdalina* was performed using 400g of each dried plant samples in 2000ml each of distilled water and methanol. The various extracts gotten were dehydrated hot water bath, weighed, filtered using Whatman No. 1 filter paper and concentrated by a rotary evaporator, and the residual extracts were finally dried. The percentage yield was obtained using dry weight, from the equation provided below. The extracts were kept and stored in refrigerator at 5 °C until use.

2.3 Percentage Yield of extract: Using the weight of the samples, the percentage yield is then calculated by:

% Yield of extract (g/100 g) = $(W_1 \times 100) / W_2$ Where W_1 is the weight of the extract residue after solvent removal and W_2 is the weight of dried plant powder.

2.4 Preparation of fermented Plant samples: Water was boiled, poured into a clean flask, left for few minutes and transferred into sterile bottles. Twenty(20g) of the leaf extract and ten (10) ml of *S. cerevisiae* culture was added to the bottle, corked, sealed tight, kept in a dark environment and left undisturbed for two (2) weeks. After the 2weeks, racking was done by draining off the clear portion into another sterile container. The sediments from the bottom of the bottle were collected and analysed for microbiological parameters. The remaining mixture in the bottle was racked again after additional 2weeks and another 1week following similar protocol. After a total of 5weeks, the contents in the bottle were left to age.

2.5 Evaluation of Physico-Chemical parameters of fermented plant extracts

2.5.1 Determination of Specific gravity: A manual hydrometer was placed inside a cylinder containing the fermenting plant extract with the weighted end down. It was then allowed to settle down and stop dipping before any reading is taken and recorded. The specific gravity is graduated along the hydrometer stem at different points for ease of reading (Awe, 2011).

2.5.2 Determination of alcoholic content: The readings from the hydrometer were used to evaluate the alcoholic content. The hydrometer was inserted into the extract and allowed to float freely without touching the sides. The reading is taken from the lower meniscus and the values used to calculate the specific gravity using the formula: $ABV(\%) = [\text{Initial Gravity} - \text{Final Gravity}] \times 131.25$ where ABV= Alcohol By Volume.

2.5.3 Determination of pH: The pH of the extracts was determined using the pH meter model PH 2601. The meter was standardized using a buffer solution prepared with a pH buffer powder of pH 4.0 at 25°C dissolved in 250ml distilled water. The electrode of the meter was immersed in a glass beaker containing the sample. The readings were got from photo-indicator of the meter

2.5.4 Evaluation of the titratable acidity (TA) of the extracts (Oladipo *et al.*, 2014): The evaluation of TA was carried out with the aid of sodium hydroxide using phenolphthalein indicator solution. About 10ml of the fermented extracts was poured into the beaker. About 5 drops of the indicator was poured into the beaker containing the extract. The phenolphthalein indicator is usually clear in an acidic solution. However, it becomes purple colour when the solution turns neutral to basic in pH. Normally, 0.1N NaOH (Normal Solution Hydroxide) is added to fermented extract in the beaker till the solution begins to change into pink colour and remains pinkish. Thereafter, the volume of the NaOH used for the titration on a milliliters graduated pipette was recorded. Titratable acidity (T.A) of fermented sample = (number or milliliters of NaOH/ Number of milliliters of juice or extract) X 0.75.

Titratable acidity is calculated in number of grams of tartaric acid per 100milliliters of juice

2.5.5 Evaluation of viscosity of the extracts: The manual viscometer was set up to allow the extracts to pass through a vertical U-tube while travel time for the extract relative to that of water was recorded. Similarly, density of the extract relative to that of water was recorded. The values were used to calculate the viscosity.

2.6 Evaluation of the microbial composition of the fermented extracts

2.6.1 *Saccharomyces cerevisiae*: Peptone water was prepared and sterilized by autoclaving at 121°C for 15 minutes and 15 Lbz. A serial dilution of between 10⁻¹ to 10⁻⁵ was then performed using 1 ml of each fermented sample. Initially, 1ml of the fermented extract was transferred into 9ml of peptone water. Then other dilutions were obtained by serial dilution until 10⁻⁵ dilution was got. Sabouraud Dextrose Agar (SDA) was prepared according the manufacturers instruction and impregnated with 0.05mg/ml of streptomycin to prevent the growth of bacteria. Ten (10)mls of the extracts were inoculated on the medium using plate method. Each dilution of the extract was inoculated into the plates in triplicates. The extracts were fermented in a period of five weeks. These culture plates were afterwards incubated at 37°C for 48 to 72 hours. At the end of incubation period, the culture plates were evaluated for colony counts.

2.6.2 Colony count: This was performed using the protocol described by Cheesbrough (2006).

Furthermore, discrete cells from the plates were sub-cultured into slants to prepare for sub culturing in other to produce pure cultures of the Yeast cells.

2.6.3 Gram Staining of *S.cerevisiae*: This was performed using the protocol described by Cheesbrough (2006). A small portion of the colony of the yeast in the slant was picked and gently smeared on a glass slide and allowed it to air dry. The smear was then heat fixed by holding the slide at one end as the glass is passed over a flame source a few times. The glass containing the smear was covered by a drop of crystal violet and allowed to stay for 60 seconds. The smear was rinsed with carefully, with water. A drop of Gram's stain was dispensed on the smear and left for 1 minute. The washing with water was performed over the smear again left for 1 minute. The smear was decolorized with 95% alcohol and washed once more with water. A drop of safranin was poured on the smear and left to stay for 60 seconds. It was further rinsed with water and left to air dry. Fungi are gram positive and will pick the stain. It was covered with a cover slip and viewed under a microscope at low power (10X) to identify the shape, structure of conidia, pigmentation and other salient features. The observation was recorded.

2.6.4 Isolation of *Lactobacillus sp* : Peptone water was prepared and sterilized by autoclaving at 121°C for 15 minutes and 15 psi pressure. A serial dilution of between 10⁻¹ to 10⁻⁵ was then performed using 1 ml of each fermented sample. Appropriate dilutions of the fermented *A. annua* and *V.amygdalina* were plated on De Man Rogosa Shape Agar (MRSA) agar and allowed to incubate for between 24 to 48 hours in an anaerobic condition at 37°C (Cheesbrough, 2006). De Man Rogosa Shape Agar (MRSA), was also prepared according the manufacturers instruction Ten (10) ml of the extracts were inoculated on the medium using plate method. The extracts were fermented a period of five weeks. The colonies were sub-cultured in fresh media by the streaking technique with the aid of a wire loop to get pure cultures. The identity of bacteria species were got using Gram staining and biochemical tests according to the method of Harrigan (2000). Subsequently, the cultures were transferred under sterile conditions into 1.5 ml Eppendorf tubes and centrifuged for 5 min at 6000 rpm. The floaty liquid was separated and cells were resuspended in sterile water. Gram staining protocol was then followed. At the end of the incubation period, distinct colonies were picked and moved into sterile broth media. Pure cultures were obtained after streaking on agar plates. These isolates were afterwards evaluated on the basis of their colony morphology, catalase and other biochemical tests and gram reaction.

2.6.5 Gram Staining: Cheesbrough (2006) technique was used for gram staining of bacteria.

A loopful of the bacterial colony was picked from the broth culture and pour small quantity on glass slide containing some sterile distilled water. A thin preparation from the mixture of water and the colony was made and fixed. A drop of crystal violet stain was poured on the smear and allowed to stay for between 30 to 60 seconds. Afterward, rapidly and carefully drain off the stain with sterile water until no presence of water is observed on the smear. Next a drop of Lugol's iodine was added and allowed for another 30 to 60 seconds before washing it off with sterile water. Next, a drop of Acetone-alcohol was poured on it to effect decolourization within few seconds and also washed with sterile water. The smear was then covered with a drop of neutral red stain and allowed for about two minutes and again washed off with water. The smear was placed on a draining rack for air- drying. Finally, the smear was examined microscopically using a x40 objective in order to evaluate the staining and to observe the distribution of materials. The second examination was performed an oil immersion objective and the out-come was recorded.

2.6.6 Biochemical tests: Indole test: The test organism was inoculated in a bijou bottle which contained about 3ml of sterile peptone water and incubated for 2 days at between 35°C- 37°C and afterwards, about 0.5 ml of Kovac's reagent

was added and gently shaken. The presence of a red surface layer indicates a positive test after 10 minutes, otherwise it is negative. Here the result was indole negative (Cheesbrough, 2006).

Oxidase: Few drops of oxidase reagent were poured on a filter paper in a sterile culture plate. Next, with the aid of sterile wire loop, the colony of the test organism as streaked on the filter paper. Any blue-purple colour noticed only within 10 seconds showed a positive oxidase test result otherwise the result is oxidase negative. In this case no blue-purple colour was observed within the 10 seconds which inferred that the test organism was oxidase negative (Cheesbrough, 2006).

Catalase: About 2-3ml of 3% H_2O_2 was poured into a test tube. With the aid of a sterile glass rod, few colonies of the test organism was picked and inserted into the test tube and observed for the presence of active bubbles or otherwise. The result showed absence of bubbles inferring a negative catalase test (Cheesbrough, 2006).

Voges Proskauer

Citrate: With the aid of a sterile wire loop, a streak was made on Slopes of Simmon's citrate agar which were prepared in bijou bottles and kept in a freezer at $2-8^{\circ}C$ and incubated at $35^{\circ}C$ for 2days. The presence of a bright blue colour indicated a positive citrate test otherwise it is negative. Equally, the result showed that the test organism was citrate negative.

Lactose utilization: here, the test was performed using a media prepared using sodium chloride(15gm), Peptone(10gm), Lactose(5gm), Phenol red (0.018gm) dissolved in 1000ml of distilled water. The test organism was introduced incubated for 2days at $35^{\circ}C$. A colour change from red to yellow showed a positive test otherwise it is negative (Pundir *et al.*, 2013).

Methyl Red test: MRVP broth was prepared in test tubes and further inoculated with 2 loopful of the test organism. The tubes were incubated for between 2-3 days at $37^{\circ}C$. Few drops of methyl red were added to the tubes and the presence of red colour indicated a positive test.

Glucose: Here, the test organism was inoculated into test tubes of 10ml glucose broth containing Durham's tube (upturned and immersed), and incubated for 1 -2 days at $37^{\circ}C$. The production of gas in the empty space in the Durham's tube indicated a positive outcome.

Tolerance of 4% NaCl: The tolerance of sodium chloride of the culture of 1% fresh overnight culture of the test organism incubated in MRS broth with 4% NaCl conc for one day was performed. the presence of growth or turbidity showed a positive 4%NaCl tests (Pundir *et al.*, 2013).

Milk Coagulation Assay: In this test 100% fresh but overnight culture of the test organism was dropped inside 10% sterile skim milk and incubated for 2 days at $37^{\circ}C$ (Chakraborty and Bhowal, 2015)

2.7 Evaluation of Antioxidant Property of plant extracts

i. Evaluation DPPH (Diphenylpicrylhydrazyl) Scavenging Activity (Hatano *et al.*, 1988): 25-500 μg of of DPPH (in 4 ml of distilled water was poured into a methanolic solution and the blend was shaken and allowed for 30 minutes at room temperature. The absorbance of the resultant solution was evaluated with the aid of a spectrophotometer at 517 nm and the standard used was Catechin (50 μg). The radical scavenging activity (RSA) was then computed as percentage of DPPH discoloration using the equation: $\% RSA = 100 \times (1 - AE/AD)$

AE = absorbance of the solution with extract; AD = absorbance of the DPPH solution without extract.

DPPH scavenging Activity = $\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$

ii. Superoxide Scavenging Activity: This was performed using a modified technique (30). The reaction mixture contained 0.2ml of NBT (1mg/ml of solution in DMSO), 0.6ml different extracts, 2ml of alkaline DMSO (1ml DMSO containing 5Mm NaOH in 0.1 ml water) in a final volume of 2.8ml. The absorbance was measured at 560nm, using UV-VIS spectrophotometer. The blank consisted of pure DMSO Instead of alkaline DMSO. The results was expressed as ascorbic acid equivalent which was used as a standard.

$\% \text{Superoxide radical scavenging activity} = (A_0 - A_1 / A_0) \times 100$

Where A_0 = Absorbance of control (blank); A_1 = Absorbance of sample

iii. Hydroxy Radical Scavenging Activity (HRSA): Hydroxy radical scavenging activities of the leaves extract, was determined using the stable radical DPPH (1, 1-diphenyl-1 picrylhydrazyl hydrate) according to the method of Blois (1958) as describe by Babalola and co-workers. The principle is based on the reaction of DPPH, and an antioxidant compound to generate hydrogen, which is reduced ($DPPH + RH \rightarrow DPPH_1 + R$). The observed colour change from deep violet to light yellow was measured at 517nm to 1ml of varied concentrations of the extract or standard, was added 1ml of 0.3mM DPPH in methanol. The mixture was vortexed, and then incubated in a dark chamber for

30minutes. Thereafter the absorbance was read at 517nm against a DPPH control containing only 1ml of methanol in place of the extract. The antioxidant activity (AA) was then calculated using the formula:

$$AA = [(A_o - A_c)/A_o] \times 100,$$

Where: A_o = absorbance without extract and A_c = absorbance with extract

Reducing Anti-oxidant power: Here, 0.025, 0.05, 0.1, and 0.25 mg/mL of pure compounds and the extracts were combined with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The combination was then incubated at 50°C for 20 min. Aliquots (2.5 mL) of 10% methanol acid were added to the combination. This afterwards centrifuged for 10 min at $1000 \times g$. The upper layer of the solution (2.5 mL) was blended with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, and the absorbance was taken at 700 nm in a spectrophotometer. Catechin was used as a standard antioxidant compound. The analysis of each assay solution was replicated three-times.

Reducing Anti-oxidant power: $A_2 - A_0/A_2 \times 100$

A_0 =Initial Absorbance of sample: A_2 =Absorbance of sample per minute after 2 minutes

2.8 Statistical Analysis of Results: Results obtained were recorded as mean \pm SEM and subjected to one way analysis of variance (ANOVA) and where significant differences exist, means were compared using Fisher's LSD method. It was performed using Statistical Analysis System at 0.05 significant level ($P < 0.05$).

3. RESULTS AND DISCUSSIONS

The physicochemical parameters revealed that specific gravity of the extracts significantly decreased ($P < 0.05$) with progression in fermentation of leaf extracts of *A.annua* and *V.amygdalina*.

The alcohol content of fermented leaf extracts of *A.annua* and *V.amygdalina* was not significantly different ($P > 0.05$) even when there was slight increase in alcohol content during the fermentation of the extracts.

The pH in the fermentation of *A.annua* and *V.amygdalina* showed a significant decrease in pH ($P < 0.05$; $**0.01$; $***0.001$) as fermentation progresses.

Temperature showed a significant increase ($P < 0.05$) with advancement in fermentation. Titratable acidity and viscosity were significantly different ($P < 0.05$; $**0.01$ and $***0.001$) (Table 1).

The in-vitro antioxidant activity of fermented *A.annua* and *V.amygdalina* indicated that HRSA% had the highest activity (Table 2).

The antioxidant activities recorded by these extracts may be related to the presence of phytochemicals such as of phytochemicals, namely terpenes, flavonoids and coumarins. flavonoid termed as chrysoprenol D, phenols and many more in the non-fermented and fermented extracts (Chauhan *et al.*, 2012). In the case of the fermented extracts, the presence of the bioactive compounds may have been facilitated by the fermentative activity of the microbes (Ooi *et al.*, 2020). The antioxidant activity of *A.annua* was also reported by Axelle *et al.* (2020). Luo *et al.* (2022) and Lang *et al.* (2019) established that the antioxidant effects of *Artemisia annua* is linked to the presence of phytochemicals, namely terpenes, flavonoids and coumarins. Flavonoid termed as chrysoprenol D.

Iwalewa *et al.* (2020), showed that the methanolic and aqueous leaf extracts of *Vernonia amygdalina* displayed antioxidant.

Plant extracts often exhibit antioxidant activity via masking of c-Jun amino terminal kinases (JNK) and nuclear factor kappa B (NF-Kb) pathways, lipogenesis control, fatty acid oxidation and triggering of AMP-activated protein kinase (AMPK) (Ahangarpour *et al.*, 2019).

Although there was no significant increase in the cells of *S.cerevisiae* and *Lactobacillus sp* in the fermented extracts for most of the period of fermentation, there was slight increase in CFU with decrease in pH (On *et al.*, 2023). This was perhaps due to their ability to use multifaceted regulatory techniques in order to adapt even in low to high pH during fermentation which may be linked to the production of various organic acids in the course of the lactic acid fermentation (Giraffa *et al.*, 2010).

The Morphological and cultural Characteristics of the isolates of *Lactobacillus spp* are presented. They are non-motile, non-spore forming, non-capsule forming gram positive rods (Table 2, Figure 2).

The Morphological and cultural Characteristics of the isolates of *Saccharomyces cerevisiae* are presented They are cream colored, oval shape, budding colonies (Table 3, Figure 1).

There were no significant differences in the CFU of *S.cerevisiae* and *Lactobacillus sp* in the fermented leaf extracts of *A.annua* and *V.amygdalina*. Although there was a slight increase in CFU AT 1/100 dilution with a drop in pH (Table 10).

The morphological and physiological characteristics of *Lactobacillus spp* and *S.cerevisiae* obtained in this study have been reported by Rahmati (2017). These characteristics enable them to adapt to variable conditions, retain viability and

effectively colonize their substrates without being adversely affected. This in turn facilitates the fermentation of the extracts thereby releasing bioactive compounds with possible antidiabetic and other related properties. In addition, these microbes have been classified as probiotics whose beneficial roles in brewing and medical sciences have been reported (Park *et al.*,2007; Kechangia *et al.*,2013 and Dilgado-Ospino *et al.*,2022).

Table 1: Physicochemical parameters of fermented extracts of *A.annua* and *V.amygdalina*

Extract/Day	SG	AC	PH	TO	TA	V	V
A.ADAY 0	1.06±0.001*	0.00±0.00ns	5.00±0.01*	30.00±0.01*	0.17±0.00*	1.5±0.00*	1.5±0.00*
A.A DAY 1	1.06±0.001*	0.2±0.01 ^{ns}	5.00±0.0*	30.0±0.03*	0.23±0.009***	1.92±0.00*	1.92±0.00*
A.ADAY14	1.048±0.001*	1.2±0.01 ^{ns}	4.59±0.02***	35±0.20*	0.332±0.023* **	2.13±0.00*	2.13±0.00*
A.ADAY28	1.03±0.001*	2.3±0.01 ^{ns}	4.43±0.00**	37.4±0.28*	0.54±0.09***	2.31±0.00**	2.31±0.00**
A.ADAY35	1.03±0.001*	4.24±0.075 ^{ns}	4.23±0.04**	39.0±0.00*	0.58±0.009***	1.96±0.00 ^{ns}	1.96±0.00ns
V.ADAY0	1.07±0.001*	0.00±0.00 ^{ns}	5.08±0.0*	29.01±0.01*	0.20±0.04*	2.00±0.01*	2.00±0.01*
V.ADAY1	1.057±0.001	0.2±0.01 ^{ns}	5.08±0.0*	29.8±0.12*	0.307±0.06***	2.15±0.01***	2.15±0.01***
V.ADAY14	1.047±0.002*	1.3±0.01 ^{ns}	4.55±0.05***	35.21±0.002*	0.465±0.009***	2.66±0.00***	2.66±0.00***
V.ADAY28	1.03±0.001*	2.5±0.01 ^{ns}	4.47±0.04**	34.77±17.04*	0.527±0.021***	2.77±0.00***	2.77±0.00***
V.ADAY35	1.03±0.002*	4.2±0.259 ^{ns}	4.6±0.03***	39.07±0.02*	0.59±0.009a***	2.5±0.02 ^{ns}	2.5±0.02ns

Values represent mean ± SD(n=3/group). Mean values with * on same column are significantly different (* p<0.05 ; ** p<0.01 ; *** p<0.001) while ns are not significant.

SG=Specific gravity; AC=Alcohol Content; pH=acidity or basicity of solution; TO=Temperature, TA=Titratable Acidity; V=Viscosity.

A.A0= *A.annua* day zero; V.A0=*V.amygdalina* day zero;

A.A1= *A.annua* day 1; V.A1=*V.amygdalina* day1; A.A14=*A.annua* day14; V.A14=*V.amygdalina* day 14
A.A28=*A.annua* day 28; V.A28=*V.amygdalina* day28; A.A35=*A.annua* day35;
V.A35=*V.amygdalina* 35

Table 2: Morphological and cultural Characteristics of the isolates of *Lactobacillus* spp

Elevation	Flat	
Configuration	Round	Colonies grow fast, flat, multidimensional
Margin	Wavy	Budding, moist, glittering, cream to tannish cream Colour
Surface	Mucoid	
Texture	Dry	
Motility	Non- motile	
spore forming	Negative	
gram's reaction	Positive	
Capsule	Negative	
colony size	0.1- 0.5mm	
Shape	Rod	

Table 3: The cultural and Morphological Characteristics of the isolates of *S.cerevisiae*

Cultural characteristics	Morphological characteristic
Colonies grow fast, flat,	Distinctive
multidimensional budding	lack true hyphae
moist, glittering , cream to tannish cream colour	egg-shaped

Table 4: Colony Forming Units (CFU/ml) of fermented leaf extracts of *A.annua* and *V.amygdalina* Using *Saccharomyces cerevisiae* and *Lactobacillus* sp respectively sp n=3

Day	Dilution	A.AF	V.AF
1	1/100	23.00±2.00 ^{ns}	22.0±2.65 ^{ns}
	1/1000	15.67±3.79 ^{ns}	17.33±5.69 ^{ns}
	1/10000	3.67±2.52 ^{ns}	1.67±0.58 ^{ns}
14	1/100	44.33±5.13 ^{ns}	44.33±5.13 ^{ns}
	1/1000	18.67±3.21 ^{ns}	18.67±3.21 ^{ns}
	1/10000	11.00±1.00 ^{ns}	11.00±1.00 ^{ns}
28	1/100	63.33± 15.28 ^{ns}	61.33± 14.57 ^{ns}
	1/1000	30.00±1.00 ^{ns}	27.33±2.52 ^{ns}
	1/10000	53.67±15.17 ^{ns}	16.67±1.53 ^{ns}
35	1/100	53.67±15.17 ^{ns}	52.33±14.98 ^{ns}
	1/1000	20.00±1.00 ^{ns}	11.67±2.89 ^{ns}
	1/10000	9.67±1.53 ^{ns}	2.33±2.52 ^{ns}

Values represent mean± SD (n=3/group). Values with same superscript (ns) are not significantly different (P>0.05)

Table 5: In-vitro Antioxidant property of Fermented and Non-Fermented *A.annua* and *V.amygdalina*

Sample	DPPH%	SOD%	HRSA%	RO%
A.AAQ	61	67.5	90.6	5.935
A.AM	60	66.5	90.4	4.119
V.AAQ	36.6	61	79.1	3.02
V.AM	60	59.7	71.9	2.376
A.AF	66.25	68.69	69.1	74.9
V.AF	40.8	37.88	41.9	40.7

A.AAQ= aqueous *Artemisia annua*

; A.AM.= Methanolic *Artemisia annua*;

V.AQ. =aqueous *Vernonia amygdalina* ; V.AM= Methanolic *Vernonia amygdalina*

DPPH = 1,1Diphenyl 2-picryl Hydrazyl;

SOD = Super Oxide Scavenging

Activity; HRSA = Hydroxy Radical Scavenging Activity; RO= Reducing Power

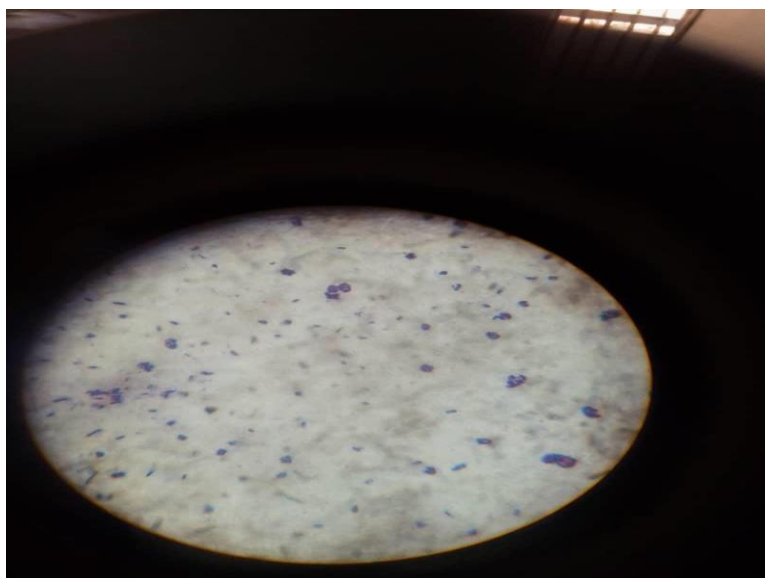


Plate 1: Slide photograph of *Saccharomyces cerevisiae*

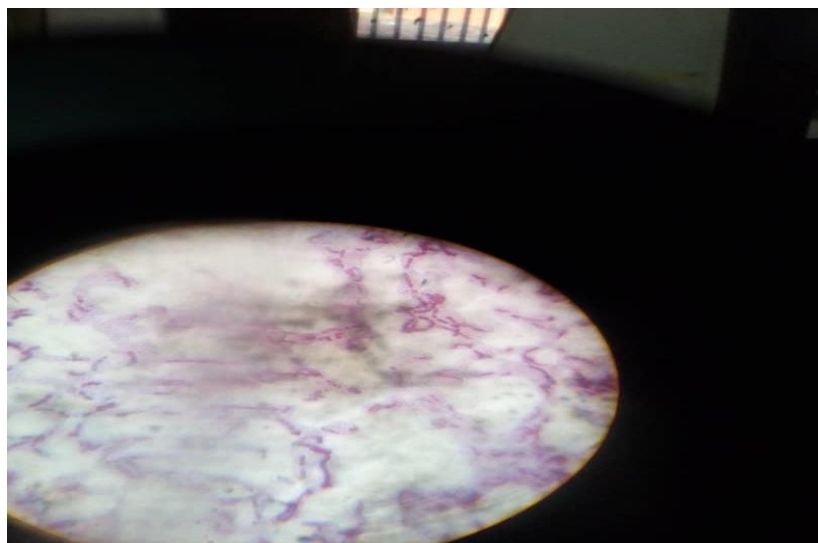


Plate 2: Slide photograph of *Lactobacillus s*

4. CONCLUSION

The investigation has revealed that fermented leaf extracts of *A.annua* and *V.amygdalina* has an enhanced antioxidant activity. The physicochemical and microbiological parameters very strongly improved this activity which is needed in food, pharmaceutical and cosmetic industries.

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