

Antibacterial Activity of Ethanolic Leaves Extract of *Andrographis paniculata* on Isolates of *Salmonella* spp. and *Escherichia coli* from Locally Processed Beverages in Umueze Nkanu West Local Government Area Enugu State Nigeria

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Abstract: The microbial contamination of locally processed beverages has been familiar among microbiological researchers who have repeatedly implicated them as the major cause of endemics due to poor processing. However, due to antimicrobial resistance and the need to discover new antimicrobial plants, Antibacterial activity of ethanolic leaf extracts of *Andrographis paniculata* against isolates of *Salmonella* spp. and *Escherichia coli* from zobo and soya milk was studied. *Andrographis paniculata* leaves were collected randomly from a local farm in Emene Enugu East L.G.A Enugu State. The leaves samples were identified morphologically, washed, air dried at room temperature and milled into powder. 39.7 g of the powder was macerated with ethanol during the extraction process. Phytochemical analysis was carried out on the extract and result showed that Saponins, Tanins, Flavanoids, Phenols, Steroids were present with Flavanoids and Saponins being in higher concentration, while Terpenoids, Alkaloids and Glycosides were absent. The zobo and soya milk samples were diluted using 10-fold serial dilution method and introduced into the already prepared MacConkey and Salmonella Shigella Agar for incubation. Isolates from zobo and soya milk were characterized, biochemically and morphologically and were identified as *E. coli* and *Salmonella* spp. Antibacterial activity of ethanolic leaf extracts of *A. paniculata* against *E. coli* and *Salmonella* spp. was determined using agar well diffusion method, and result showed that there was no antibacterial activity of ethanolic leaf extracts of *A. paniculata* against *E. coli* and *Salmonella* spp. However, absence of Terpenoids, Alkaloids and Glycosides exhibited non-comparable activity with the positive control (ciprofloxacin).

Keywords: *Andrographis paniculata*, Zobo, Soya milk, Antibacterial activity, Terpenoids, Alkaloids and Glycosides.

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INTRODUCTION

Locally processed beverages are unregulated drinks, commonly referred to as artisanal, unrecorded, illicit, or illegal drinks in a community. A common attribute of these kinds of products is that they are produced outside government regulation without any rules or regard to standard food safety guidelines. They could be home-made, surrogate, and counterfeit alcoholic beverages that predispose people to hazardous

substances example zobo drinks. The lack of extensive regulations for most local products can affect public health because these products often contain compounds that are fermented by microorganisms, which may lead to food infection, intoxication, renal failure and other illnesses. Sometimes a product may be regulated at the production stage but not during sales where the products can be displayed and sold under direct sunlight or inappropriate temperatures (Ogungbemi *et al.*, 2017).

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Man has always been challenged by microorganisms either through direct contamination of the food he eats or by direct contact from fomites and there is no indication that it would change anytime soon. It is the contamination of food that is most problematic because, consuming contaminated food results in diseases such as diarrhea and typhoid fever which are caused by microbial pathogens of various origins but most importantly those belonging to the *Enterobacteriaceae* family of which *Escherichia coli* and *Salmonella* species are prominent members. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide. *Salmonella* and *Campylobacter* are the most frequently isolated food borne pathogens, and are predominantly found in poultry, eggs and dairy products.

Andrographis paniculata has been considered as a medicinal shrub and used as a medicinal plant in the remote areas of Bangladesh. The extracts prepared from *A. paniculata* stem showed better antibacterial activity against all the strains of bacteria *i.e.*, *E. coli*, *S. typhi*, and *B. Subtillis* (Polash *et al.*, 2017). *Andrographis paniculata* is an economically important herb of the *Andrographis* genus. It is an erect and branched and annual flowering herb. This herb grows well in hedgerows throughout the plane lands, hill slopes, waste ground, farms, moist habitat, seashores, and roadsides. It can also be grown in the garden as well. For their excellent development, moist and shady places, forests, and wastelands are preferable. It is a salt-sensitive plant; therefore, its growth is limited under stress conditions, particularly salinity stress that drastically affects plant growth and crop productivity (Hossain *et al.*, 2021). This plant, under cultivation, can reach up to a height of 30 to 110 cm. Its stem is dark green, 30–110 cm in length, 2 to 6 mm in diameter, quadrangular with longitudinal furrows and wings at angles of the young parts, slightly enlarged at the nodes. The leaves are dark green, glabrous, 2–12 cm long and 1–3 cm broad, opposite, decussate, lanceolate, entire margin, and venation pinnate; the petiole is very short. The flowers are small; consist of five linear particle calyxes, narrow tube, and about 6 mm long white corolla with rose-purple spots on the petals. December to April is the flowering and fruiting period. The fruits are small 2-celled odourless erected capsules, 1–2 cm long, 2–5 mm wide, linear-oblong, acute at both ends and compressed. The leaf taste is intensely bitter, and seeds are numerous, sub-quadrate and yellowish-brown (Hossain *et al.*, 2021).

This annual plant belongs to the *Acanthaceae* family and is commonly known as “*King of the bitters*”. *Andrographis. paniculata* contains steroids, phenols, terpenoids, alkaloids, saponins, flavonoids were the active compounds present in the plant. The medicinal value of this plant is due to the presence of active ingredients andrographolide and neoandrographolide which are derivatives of

diterpenoids. The presence of various chemical constituents in the aerial parts of the *Andrographis paniculata* are andrographolide, which is diterpene lactone, colourless, crystalline, bitter in taste. It is native to India and Sri Lanka and widely found in Southern and Southeastern Asia, including Bangladesh, China, Hong Kong, Indonesia, Malaysia, Myanmar, Philippines, Thailand and Nigeria. Usually, the aerial parts, roots or leaves of *A. paniculata* are used separately. These plant parts are used traditionally as powder, infusion, or decoction form either alone or in combination with other medicinal plants for the treatment of dysentery, peptic ulcer, gastro enteritis, gonorrhoea, respiratory tract infections, scabies, boils, chronic and seasonal fevers, griping, irregular bowel habits, loss of appetite, alopecia, diabetes, jaundice, dyspepsia, cough, oedema, liver complaints, herpes, and skin infections (Polash *et al.*, 2017).

MATERIALS AND METHODS

Study Design

The already prepared fresh zobo and soya milk beverage samples were randomly collected from ten local shops in Umueze Nkanu West L.G.A. Enugu State. This was to assay the antibacterial activity of *Andrographis paniculata* extracts on some enteric organisms present in the locally produced beverages and if effective can serve as adjunct treatment during the production process. The study was designed, to take place during the dry season when there is scarcity of water.

Ethical Consideration

Ethical consent was sought from zobo and soya milk sellers but they refuse to give full identities of themselves and manufacturing addresses of their products out of fear.

Collection and Transportation of Plant Samples

Fresh leaves of *Andrographis paniculata* from a local farm in Emene Enugu East L.G.A Enugu State were collected using stainless clipper that was sterilized in the hot air oven and were transferred into sample plastic bags with zip lids. The lids were sealed immediately the plants had been placed in the plastic bags. The samples were transported to Microbiology laboratory of Enugu State University of Science and Technology Enugu State Nigeria immediately after collection and the leaf sample was identified by a plant taxonomist Prof J.C Okafor of Enugu State University of Science and Technology.

Collection and Transportation of Zobo and Soya Milk Samples

The already prepared fresh zobo and soya milk beverage samples were randomly sourced from ten local shops in Umueze Nkanu West L.G.A. Enugu State. The samples were obtained and placed in an iced cooler and immediately delivered to Microbiology

laboratory of Enugu State University of Science and Technology Enugu State Nigeria for analysis.

Ethanol Extraction of *Andographis paniculata* Leaves

The extraction was carried out as done by (Gurupriya and Cathrine, 2016). Fresh leaf of *Andographis paniculata* weighing 39.7 g was washed in running water to remove unwanted materials. The leaves were air-dried for two weeks and ground into fine powder. 36.9g of ground sample was weighed and soaked in 270 milliliters of 90% ethanol solution (BDH, England), stirred and allowed to stand for 24 hours. The suspension was filtered using Muslin bag followed by Whatman No. 42 filter paper. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55°C. The concentrated extract was stored in a labeled sterile screw capped bottle at 2°C.

Preparation of Different Concentration for the Extract

The preparation of different concentration for the extract was carried out using the method described by (Geetha *et al.*, 2017). 500mg/ml, 300mg/ml and 100mg/ml concentrations of the crude extract of ethanol were prepared by dissolving 5g, 3g and 1g of the extract in 10ml of 5% DMSO respectively.

Isolation of Some Enteric Organisms from Zobo and Soya Milk Beverages

This was carried out using a method described by (Ekanem, 2018). 1 ml of zobo and soya milk beverages was suspended in test tubes containing 9 ml of sterile distilled water which was properly mixed and allowed to stand for 1 minute. The suspensions were serially diluted in ten-fold and thereafter streak plated on Salmonella Shigella Agar (SSA), MacConkey agar, Nutrient agar and Eosin Methylene Blue agar (EMB) and were incubated for 24 hours at 37°C. After incubation, discrete colonies were severally sub

cultured on Nutrient agar and incubated for 24 hours at 37°C to obtain pure culture.

Identification of Isolates from Zobo and Soya Milk Beverages

The isolates were identified according to (Cheesbrough, 2000). The identification was performed after incubation by morphological description of colonies on the selective media (MCA, SSA and EMB) of plates 10^{-3} , and 10^{-4} , which showed discrete colonies of *E. coli* and *Salmonella* spp. Isolated colonies were transferred into agar slants to obtain stock cultures of the isolates and stored at 40°C in refrigerator for further study. Other test carried out were Gram staining, Indole, Voges proskauer tests, Catalase, Oxidase, Urase, Glucose, Lactose, Methyl red and Citrate utilization.

Antibacterial Activity of *Andographis paniculata* leaves extract on *E. coli* and *Salmonella* spp.

The bioassay was carried out by agar well diffusion method as done by (Ramya and Devi, 2011). The culture media used for the assay was the Mueller Hinton agar. Micro-dilutions of fresh cultures of *E. coli* and *Salmonella* spp. were inoculated on the surface of the Mueller Hinton agar using a bent glass rod. Six plates in total were used to carry out this assay; three plates were used for each of the two isolates. Agar wells of 6mm diameter were cut out using a sterile cork borer and filled with the plant extract. Ciprofloxacin was used as the positive control while dimethyl sulfoxide served as the negative control. The plates were incubated for 24 hours at 37°C and zones of inhibition were measured using a ruler calibrated in millimeters.

RESULTS

The phytochemical analysis of ethanol extracts of *Andographis paniculata* leaves was shown in Table 1.

Table 1: Phytochemical of *Andographis Paniculata* Leaves

Phytochemicals	Estimated Concentration
Alkaloids	-
Phenols	+++
Glycosides	-
Tannins	++
Saponins	++
Terpenoids	-
Steroids	++
Flavonoids	+++

KEYS

-	:	Absent
+	:	Scanty
++	:	Moderate
+++	:	Abundant

The morphological characteristics of the *Salmonella* spp and *Escherichia coli* from zobo and soya milk beverages are shown in Table 2.

Table 2: Morphological Characteristics of the Salmonella spp and Escherichia coli

Bacterial Isolate	Macroscopic Characteristics	Microscopic Characteristics
<i>Salmonella</i> spp	Consisted of smooth, opaque with black-centered colonies Salmonella-Shigella agar	Consisted of Gram negative single short rods which appeared pink.
<i>Escherichia coli</i>	Consisted of thick, metallic green sheenon Eosin Methylene Blue agar and also appeared mucoid bright pink colonies on MacConkey agar medium.	Consisted of Gram negative single short rods which appeared pink.

The biochemical characteristics of *Salmonella* spp. and *Escherichia coli* from zobo and soya milk beverages are shown in Table 3.

Table 3: Biochemical Characteristics of Salmonella Spp. and Escherichia coli

Bacterial isolate	Gram Stain	Indole test	Catalase test	Oxidase test	Urease test	Citrate Utilization test	Methyl red test	Glucose fermentation test	Lactose fermentation test	Voges-p roskaur test
<i>Salmonella. spp.</i>	-	-	+	-	-	-	+	+	-	-
<i>Escherichia coli</i>	-	+	+	-	-	-	+	+	+	-

KEYS

- : Negative
- + : Postive

The antibacterial activities of *Andrographis paniculata* leaves extract on *Salmonella* spp and *E. coli* from zobo and soya milk beverages are shown in Table

4. Ethanol extracts of *Andrographis paniculata* leaves showed no activity against the isolates of *E. coli* and *Salmonella* spp.

Table 4: Antibacterial Activity of Andrographis paniculata leaves extract on Salmonella spp. and E. coli

S/N	Extract Concentration (g/ml)	Zones of Inhibition	
		<i>E. coli</i>	<i>Salmonella spp.</i>
1	500	-	-
2	300	-	-
3	100	-	-
4	Ciprofloxacin	14mm	20mm

DISCUSSION

Phytochemical analysis of ethanol extracts of *Andrographis paniculata* leaves were carried out and result showed that Saponins, Tanins, Flavanoids, Phenols, Steriods were present with Phenols and Flavanoids being in higher concentration, while Terpernoids, Alkaloids and Glycosides were absent (Table 1). This might be the reason why this study has contradictory results in regards to work of (Polash *et al.*, 2017) on Investigation of the Phytochemicals, Antioxidant, and Antimicrobial Activity of the *Andrographis paniculata* Leaf and Stem Extracts. Phytochemicals which are the active components of plants may be deficient in certain sub varieties of plants or simply due to geographical origin of the plant.

Experiments were also conducted on already prepared fresh zobo and soya milk beverage samples randomly collected from ten local shops in Umueze Nkanu West L.G.A. Enugu State. The enteric organisms

isolated from the zobo and soya milk were morphologically identified as *Escherichia coli* and *Salmonella* spp. using standard morphological identification techniques (Tables 2 & 3). This result agreed with the work of (Awol *et al.*, 2021) who conducted a research on Prevalence of *Salmonella enterica* serovar Typhi infection, its associated factors and antimicrobial susceptibility patterns among febrile patients at Adare general hospital, Hawassa, Southern Ethiopia.

The antibacterial activities of *Andrographis paniculata* leaves extract on the isolates were carried out and the result showed that there was no antibacterial activity of *A. paniculata* against both isolates. The finding does not agree with Polash *et al.*, (2017), as the ethanolic (70%) and water (30%) leaf and stem extract of *A. paniculata* showed activity against *E. coli* and *Salmonella typhi* in their study. Shalini and Narayanan, (2015), confirmed activity of methanolic leaf extract of

A. paniculata against *E. coli* and *Salmonella* spp., which is contrary to the outcome of this study. The review hypothesized the non-activity of *A. paniculata* as identified in previous literature, thus outlining that, possible reasons may stem from the absence of vital antimicrobial phytochemicals (Polash *et al.*, 2017). In this study, the Phytochemical analysis carried out on the extract showed that Terpernoids, Alkaloids and Glycosides were absent. This might be the reason why this study has contradictory results in regards to previous studies.

CONCLUSION

This study showed that there was no antibacterial activity of *Andrographis paniculata* against the test organisms. The absence of terpernoids, alkaloids and glycosides from phytochemical constituents of ethanol extracts of *Andrographis paniculata* leaves contributed to non antibacterial activity against the test isolates of *Salmonella* spp. and *Escherichia coli*

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