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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *Tricalysia chevalieri K. KRAUSE* LEAVES EXTRACT

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ABSTRACT

An assessment of phytochemical composition and antimicrobial activity of methanol leaf extract of *Tricalysia chevalieri* was evaluated for the presence of phytochemicals. The bacterial pathogens used were subjected to biochemical tests for proper identification and antibacterial susceptibility of the crude extracts against clinical isolates of *Escherichia coli* and *Klebsiella pneumoneae* were determined at varying concentrations 200mg/ml 100mg/ml 50mg/ml 25mg/ml using agar well diffusion method. The MIC and MBC of the crude extracts were also determined. The result of Phytochemical screening revealed the presence of Saponins, Tannins, Steroids, Glycosides, Phenols and Flavonoids in the crude extracts. The methanol leaf extract exhibited good activity against the test organisms. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the leaf extract ranges from 50-100mg/ml for *Escherichia coli* and *Klebsiella pneumoneae* 25-200mg/ml respectively. The extract showed significant inhibition against all the test organisms.hence bactericidal in its effects. This study concludes that the methanol leaf extract of *Tricalysia chevalieri* contains bioactive components that have broad spectrum antimicrobial properties that can be used as a good source of drugs to cure Dysentery and Pneumonia.

Key words: *Tricalysia chevalieri,* Antimicrobial susceptibility, Phytochemical composition, Bacterial isolates.

INTRODUCTION

Tricalysia chevalieri belongs to the family Rubiaceae a large African and Asian genus. Many of the species being on the borderline between shrubs and trees (Hutchinson *el al.,* 2014). It is found in Coppices on ironstone and rocky lands, fringing forest of Guinean and Sudano-Guinean savannahs and also found in Gombe State, Nigeria. The plant is distributed from Senegal to Cameroon and as far as Sudan and Nigeria (Arbonnier, 2004). It is commonly called Burugali in Hausa language. The species found in the savannah regions of Nigeria includes *Tricalysia chevaleiri*. The plant is used for medicinal purposes, as well as a utensil for making soups in Northern Nigeria. Recently, attention was drawn towards leaf extract and biologically active compounds isolated from medicinal plants. More so, the use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antimicrobial, antifungal and antiviral agents with significant activity against pathogenic microorganisms (Kumar *et al.,* 2017).In Nigeria, Labe, 2020 reported that the leaves of *Ceiba pentandra* are highly medicinal, and more efforts are required to conserve or

protect them. In Ghana, traditional medicine, especially herbal medicine, provides many citizens with affordable healthcare services (Gilchrist *et al*., 2018). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils as well as tannin. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Oloninefa *et al.,* 2018). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide (Oloninefa *et al.,* 2018). The objective of the study was to screen for the presentce of phytochemical and antibacterial activities of *Tricalysia chevalieri* leave extract.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The *Tricalysia chevalieri* plant was collected from Kanawa Forest Reserve in Yamaltu Deba Local Government, Area in Gombe State, North-Eastern Nigeria. The plant material was identified and authenticated by a specialist, given voucher number and deposited at the herbarium of Department of Botany, Gombe State University, Gombe, Nigeria (Figure 1). Fresh leaves were collected and shade dried. The dried leaves were grounded to powder. Sixty gram (60g) of the powdered sample of the plant was weighed on electric balance and put into labelled conical flasks containing 400ml of methanol. The mixture was placed on a rotary shaker and agitated for three days and then filtered. The filtrate was put into another flask, and heated on water bath to recover the extract. (Bhojwani and Dantu, 2013).

Figure 1. *Tricalysia chevaleiri*

Phytochemical Screening of Leaf Extract

The qualitative phytochemical composition of the extracts of the plant materials were analyzed for the presence of Alkaloid, Saponin, Steroids, Tannin, Flavonoid and Glycosides according to standard methods of Aiyelaagbeand (2009).

Detection of Cardiac Glycosides

Extracts were hydrolysed with dilute 1% hydrochloric acid solution, and then subjected to test for glycosidesusing the Modified Borntrager's test. Extracts were treated with ferric chloride solution and immersed in boiling water for 5 minutes.

The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of red colour in the ammonical layer indicates the presence of glycosides (Tiwari, *et al.,* 2011).

Detection of Saponins

This was done by the Froth Test and Foam test. In the Froth test extracts were diluted with distilled water to a 20mlvolume. This was shaken for 5 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. In the Foam test, exactly 0.5 g of the extract was shaken with 2ml of water. If the foam that was produced persists for ten minutes, this indicates the presence of saponins (Sofowora, 1993).

Detection of Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue black or brownish green indicate the presence of tannins (Evans, 2002).

Detection of Steroids

5 drops of concentrated H_2SO_4 was added to 1 mL of extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts (Sofowora, 1993).

Detection of Phenol

To 1ml of the leaf extract 2ml of distilled water was added followed by two drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols (Evans,2002).

Detection for alkaloids (Mayer`s Reagent Test)

To 2ml of the extracts, 2-3 drops dilute Hydrochloric acid was added to acidified the sample, 2ml of Mayer`s reagent i.e. mercury chloride ($HgCl_2$) and potassium iodide (KI) in distilled water was added. Formation of white to yellow precipitate indicates the presences of Alkaloids (Evans,2002).

Biochemical Tests and Identification of Selected Bacterial Pathogens

The test organisms (*E. coli and Klebsila pneumonia*) were collected from Federal Teaching Hospital, Gombe and were authenticated by Bochemical tests. The test organisms were aseptically sub cultured on nutrient agar and thereafter double disc synergy test (DDST) method was performed to identify beta lactamase producing organisms. The colonies were further sub cultured to obtain pure culture as described by Cheesebrough (2006).

Standardization of Inoculum

Using inoculation platinum wire loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matchedthe turbidity of the 0.5 McFarland Standard (CLSI, 2012).

Preparation of Turbidity Standard

Adopting the method described by CLSI (2012), one percent (1% v/v) solution of Sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 into 99ml of distilled water. One percent (1%w/v) solution barium chloride was also prepared

by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water, then 0.6ml of the barium chloride solution was combined with 99.4ml of Sulphuric acid solution to yield 1% w/v barium sulphate suspension. The turbid solution formed was then transferred into the test tube as the standard for comparison CLSI (2012).

Preparation of Stock Solution of Extracts

A 200 mg/ml concentration of the extracts were constituted by dissolving 0.2 g in 1 ml of 20% dimethyl sulfoxide (DMSO) and serial dilutions was made (Esimone *et al.,*2003).

Sensitivity Testing

This assay was conducted using agar-well diffusion method (Esimone *et al.,*2003) as described below; A 200 mg/ml concentration of the extracts were constituted by dissolving 0.2 g in 1 ml each of 20% v/v dimethyl sulfoxide (DMSO) and 2-fold serial dilutions were made. A single colony of the test isolate was suspended in 2mls of sterile Muller Hinton agar. The suspension of the isolate was standardized as stated previously, and used to inoculate the surface of the Muller Hinton agar and the excess fluid was drained into disinfectant jar. The inoculated agar surfaces were allowed to dry and the plates were appropriately labelled.

Using a cork borer, four wells of 6mm in diameter were bored in the inoculated Muller Hinton agar. Using a micropipette, 200=50µl of each concentration of the test extracts were delivered into each well. The plates were left on the bench for 30 minutes to allow the extracts to diffuse into the agar. Thereafter, the plates were incubated at 37°c for 24 hours. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with meter ruler to the nearest whole millimeter. Each test was carried out twice and the mean inhibition zone diameter were recorded to the nearest whole millimeter.

Minimum Inhibitory Concentration (MIC) of the Crude Extract.

Minimum inhibitory concentration of the extract was determined using the tube dilution method (Andrew. 2001). Dilution of the plant extracts was incorporated in nutrient broth 1:1 ratio initial rough estimates of the MIC values of the plant extract against the test organism was estimated to determine the range of MIC values. Consequently, the following concentrations were prepared for each extract, using the dilution formula 200mg/ml, 100mg/ml ,50mg/ml 25mg/ml. In addition, 0.1ml of standard suspension of the test organism was added to each tube. The tubes were incubated at 37°c for 24hours. A tube containing extract and growth medium without inoculum was included which served as control. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extract showing no growth is regarded as minimal inhibitory concentration (MIC)

Minimum Bactericidal Concentration (MBC) of Crude Extracts

The minimum bactericidal concentration (MBC) was determined by sub culturing the last test dilution that showed visible growth (turbidity) and all others in which there was no growth on a fresh extract solid medium.

Zero-point one milliliter (0.1ml) from these concentrations that showed no visible growth was inoculated into 9 ml recovery Nutrient Broth (Nutrient broth containing 3% v/v Tween 80). and incubated at 37° C for further 24 hours. The least concentration of extracts that showed no single or with lowest bacterial colonies was taken as the minimum bactericidal concentration MBC (Bergen *et al.*, 2010).

RESULTS

Phytochemical analysis of the leave extract of *Tricalysia chevalieri* revealed the presence of secondary metabolites such as Saponins, Flavonoids, Tannins, Glycoside, Steroids, and Phenol while Alkaloids was not detected (Table 1).

The Result of Antimicrobial activities of the methanol leaf extract of *Tricalysia chevalieri* against the test organisms were shown in Table 2. The zones of inhibition of the isolates are the function of antimicrobial activities of the extract. The activities of the leaf extract were shown to be concentration dependent. The extract showed significant inhibition against all the test organisms, hence bactericidal. However, Dimethyl Sulfoxide DMSO used as control were inactive against the microorganisms.

Table 1: Phytochemical Screening of Methanolic leaves extract of *Tricalysia chevalieri*

Phytochemical components	Methanol
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Steroids	+
Phenol	+
Glycosides	+

Key: + = present, - = absent

Table 2: Antimicrobial activity of Methanolic leave (zone of inhibition) extract of *Tricalysia chevalieri* against the tested organisms

Concentrations	200mg/ml	100mg/ml	50mg/m	l25mg/ml
Test Bacteria	**Zones of growth inhibition (mm)**			
E. coli	18	10	11	7
Klebsiella pneumoniae	15	9	10	6

Note: The values are the averages of two measurements across each zone of inhibition and in Duplicates

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 3 shows the result of MIC and MBC determination on the test organisms. The MIC and MBC for *E. coli* are 50 and100mg/ml and 25-200mg/ml for *Klebsiella pneumoniae* respectively. The extract displayed antimicrobial activity against all tested microorganisms which shows bactericidal effects.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanol leaves extracts of *Tricalysia chevalieri* on tested organisms

EXTRACT		
Test Organisms	**Methanol (mg/ml)**	
	MIC	**MBC**
E. coli	50	100
Klebsiella pneumoniae	25	200

Note: The MIC are the averages of duplicate readings.

DISCUSSION

The results of the phytochemical analysis revealed that the extracts were positive for Phytocompounds like Tannins, Sapoinis, Flavonoids, Phenol, Steroids, Glycosides; These active phytochemical components are known for their medicinal activity/biological activities as well as their physiological actions; as such they determine the therapeutic potentials of all medicinal plants (Panda and Kar, 2007 ; Steven *et al*.,2019).. This is in line with the works of (Mbengui *et al.,* 2013) who also confirmed the presence of tannins, saponins, flavonoid, phenol and steroids in *Terminalia catappa* leaves. Tannin, Alkanoid and Saponin have been reported to inhibit bacterial growth and provide protection to plants mycoses (Mbengui *et al.* 2013). Alkaloids were not detected in the plant part which is an indication that the plant is of pharmacological importance.

Flavonoids are an integral phytochemical constituent of higher plants. They have antioxidant potentials hence could offer protection against heart disease and cancer probably by enhancing the body defence against pathology induced free radicals' generation as opined by Al-Humaid *et al.,* (2010). Tannin containing plant extract are used as astringents against diarrhea, as diuretics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals. Phenols are famous group of secondary metabolites with wide pharmacological activities. This includes anti- ulcer, anti inflamatory, antioxidant, cytotoxic, antitumor, antispasmodic and antidepressant activities (Ghasemzadeh *et al.*, 2010; Silva *et al.*,2007). Saponins are a class of chemical compounds found in particular abundance in various plant species. They are used in the manufacture of of shampoos, insecticides, various drug preparations and synthesis of steroidal hormone (Okeke and Nwachukwu , 2009).Prevoius studies have provided enormous evidences exposing that they have many health benefits.They have been reported to have antioxidant,anti-inflammatory,anti-hyperplasia,antimicrobial,and analgesic activities (Guclu-Ustundag and Mazza, 2007). Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity (Snehal and Jignasha (2015). Glycosides are naturally occurring substances and have outstanding therapeutic potential and clinical utility. Emerging results showed that numerous glycosides isolated from various plants possesd marked anticancer activity against a variety of cancer cell lines (Haroon *et al.*, 2019).

Medicinal plants are important sources of potentially useful structure for the development of new chemotherapeutic agents. Many reports are available on the antimicrobial, antifungal, antiviral and anti-inflammatory properties of plants (Behera and Misra, 2005; Govindarajan *et al.,* 2006; Mohammed ae al.,2016). Several studies have been conducted in the past that focus on the antimicrobial properties of herbs, spices and their derivatives such as extract and decoctions (Alma *et al.,* 2003). Some of these observations have helped in

identifying the active constituent responsible for such activities and in developing drugs for therapeutic use in humans. These secondary metabolites have been established to be frequently responsible for the antimicrobial properties of most medicinal plants (Esimone *et al.,* 2003; Dewanjee, 2008). Thus, the presence of the above bioactive component may account for the high antimicrobial activity of the methanol leaf extract of *Tricalysia chevalieri* against some selected microorganisms. The extract shows good activity against pathogenic microorganisms which include *E. coli* and *K.pneumoniae.*

Plant based products in some Saudi Arabian herbal plants have been effectively proven for their utilization as sources of antimicrobial compounds (Abdelaaty *et al* 2017). Methanol extract of *Tricalysia chevalieri* exhibited a strong antimicrobial potential. The MIC and MBC for *E. coli* are 50 and100mg/ml and 25-200mg/ml for *Klebsiella pneumoniae* respectively. The result showed that the extract at (200mg/ml) was most effective against *E. coli* (50mg/ml), *K.pneumonia* (25mg/ml). It was observed that the antibacterial activity increased with concentration (Table 2). In addition, the fact that treatment of infections caused by microorganisms such as *E. coli* and *K. pneumonia* are increasingly becoming difficult further strengthens the importance of finding and the need for a continues research for chemotherapeutic agents.

The result of the present investigation clearly reveals Bactericidal properties and antimicrobial importance of *Tricalysia chevalieri* leaf extract to cure diarrhea and Pneumonia and suggests that this plant could be exploited in the management of more diseases caused by the microorganisms in humans.

CONCLUSION

This study concluded that the leaf extracts of *T. chevalieri* contains the presence of phytochemicals such as Flavonoids, Saponins, Steroids, Phenols and Glycosides while Alkaloids was not detected at the leaves extract. The antimicrobial activities of the methanol leaf extract of *T. chevaliri* against test isolates *E. coli* and *K. pneumoniae*. are concentration dependent. The extract showed significant inhibition against all the test organisms hence bactericidal in its effect. The low values of MIC and MBC for *E. coli* are 50 and100mg/ml and 25-200mg/ml for *Klebsiella pneumoniae* recorded for the methanol leaf extract of *T. chevaliri* suggests that this plant can be used as a good source of drugs to cure Dysentery and Pneumonia. Further studies should be carried to isolate, purify and characterize the active constituents responsible for the activity of this plant.

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