Direct RESEARCH JOURNALS

Direct Research Journal of Health and Pharmacology
Vol.8 (1), pp. 7-13, February 2020
ISSN 2449-0814
DOI: https://doi.org/10.26765/DRJHP78098127
Article Number: DRJHP78098127

https://directresearchpublisher.org/drjhp/

Full Length Research Paper

Phytochemical screening and antimicrobial activity of ethanolic leaves and stem bark extract of Jatropha tanjorensis

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Received 2 January 2020; Accepted 20 January, 2020

The leaves and stem bark of Jatropha tanjorensis was extracted using ethanol and water for phytochemical screening and antimicrobial activities using standard method. The phytochemical screening revealed the presence of some bioactive component that possessed antimicrobial properties. Various chemical tests showed the presence of glycosides, flavonoid saponins, alkaloid, steroids, terpenoid and tannins while reducing sugar and anthraquinones were absent when tested. The effect of ethanolic and aqueous extract on some pathogenic bacteria strain showed such as staphylococcus aureus, Escherichia coli, shigella, salmonella typhi and streptococcus showed that the plant can be used to treat infections caused by bacteria. The ethanolic extract was more effective covering nearly the entire spectrum of the organism with the zone of inhibition ranging from 7.00 mm, 5.40 mm, 5.40 mm, 5.10 mm, 4.15 mm, and 3.99 mm against salmonella typhi, staphylococcus aurenus, Escherichia coli, shigella spp and streptococcus spp. The aqueous and ethanolic extract showed antibacterial activity but the significant antimicrobial activity was showed by ethanolic extract only against S/typhi and S/aureus while moderate activity against E coli strep spp. and shigella. The MICs of the ethanolic extract against the test bacteria were high and correlates with the sensitivity test result while the effectiveness of the crude extract confirmed its use in traditional medicine to treat cardiovascular disease and also to build up blood levels in physiological condition like pregnancy and menstruation when there is drop in hemoglobin and package cell volume.

Keywords: Antimicrobial, ethanolic, phytochemical, screening

INTRODUCTION

Immense benefit has been derived by mankind from the use of medicinal plants in disease management because they are relatively safer, more affordable and sometime offer more therapeutic value than synthetic drugs (UNESCO, 1998). The increased discovery of medical plants has demanded the increase scrutiny of their

bioactivity so as provide data that will help physician and patient to make wise decision before use. Natural products, principally medicinal plants have long been prescribed in traditional medicine for centuries for therapeutic value has continued to represent source of new effective medicine. Besides evidence from epidemiological studies suggest that high consumption of fruit and vegetable is linked to the reduce risk of development most oxidative stress-induced disease (Dan *et al*., 2008; Wasson *et al.,* 2008). Examples of such disease include cancer diabetes mellitus, protein energy malnutrition (PEM), cataract infection and other degenerative disease of aging (Dan *et al*., 2008; Wasson *et al*., 2009; Omoregie and Osagie, 2011).

Over the past two decade there has a lot of interest in the investigation of natural material as source of new antibacterial agents (Bonjar *et al*., 2003) according to the world health Organization (WHO), medicinal plants would be the source to obtain a variety of drug and active compounds. Therefore such plant should be investigated to better understand their properties, safety and efficiency (EIlof, 1998). This system of medicine supports the need of more than 70% of population residing in the rural areas. Besides the demand made by these system as the raw material plants made by the modern pharmaceutical industries have also increase (Bhattacharjee, 2001).

Plant and extract have formed important position in modern medicine due to their chemical and content found in natural form. Their secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. Microorganism has the genetic ability to transmit and acquire resistance to antibiotics and have become a global health problem.

The vast majority of antibiotics used today are produced by microorganism, yeast or fungi which belong to the vegetable kingdom.

Higher plants mainly produce antimicrobial compound to their defense mechanism against infection constituting cellular metabolism. Use of plant as traditional health remedies is very popular and important for 80% of the world population in Africa, Asia, Latin America and Middle Eastern countries. Their use is reported to have minimal side effects (Bibitha *et al*., 2002; Maghrani *et al*., 2005; Doughari, 2006). In recent years, pharmaceutical companies have spent considerable time and money in develop therapeutics based upon natural produce extracted from plant (Ben Sassi *et al*., 2007). The rising incidence of multi drug resistance amongst phatogenic microbes has further necessitated the need to search for newer antibiotic source (Veronica *et al*., 2006). Because of its abundant and widespread availability, this study sets out to investigate the antimicrobial activity of jatropha tanjorensis.

Although there are some method of phytochemistry already established but considering the vastness of this field and continuous discovery of closely relate component thus there are many unidentified constituent of a medicinal plant. Therefore the purpose of this research work is to identify constituents of this plant (jatroensis tanjorensis) and its quantity.

Several works carried out on the leaves extract of jatropha tanjorensis reveals that the leaf extract of jatropha tanjorensis possesses the phytochemical ingredient capable of lowering blood cholesterol level and might be useful in the treatment of cardiovascular disease caused hyghperlipidemia. Also according to the work of Omoregie and Osagie, (2011) it was found that the extract of jatropha tanjorensis can be used in building up the blood level in physiological condition like pregnancy and menstruation when there is a drop in the hemoglobin level and package cell volume PVC. This becomes great use in the rural communities where there are inadequate healthcare. Similarly for the anti-microbial activity test usually measured in term of minimum inhibition concentration (MIC) (Cody *et al*., 2000) reported that the antibiotic test of the methanol leaf extract on staphylococcus aureius, bacillus cereus E coli and bacillus substitis were given as 5.00,mg/ml .10.00mg/ml respectively. There zones of inhibition ranges between 10-12 mm. The objective of this research is establishing the potential ability of the stem bark extract of *jatropha tanjorensis* against microbial pathogens. This will in turn add to the already existing literature of antimicrobial producing plants that have been widely used in the treatment of disease.

MATERIALS AND METHODS

Collection of sample

The collection of the leaves and stem bark of the plant (Asibiti kusa) was carried out at Zuru and Aliero local government area respectively of kebbi State Nigeria. The leaves collected were air-dried at room temperature and were later pounded and sieved into a fine powder to increase its surface area for more interaction with the solvent in other to get reasonable amount of the extract. A concentrated ethanol solution was used for the solvent extraction and distill water was used for the aqueous extraction. The pounded leaves were soaked at room temperature for 3 days and the resulting mixture was filtered was distilled under normal atmospheric condition to evaporate the solvent and the extract removed.

Preparation of sample

The leaves and stem bark were air dried for 5 days and 13 days respectively and grounded into a fine powder. 10 grams of each of the powdered sample were soaked into 100 ml distilled water and allowed for 72hours and the mixture was filtered with whatsman filter paper.

The residues were discarded and the filtrate was used for qualitative for phytochemicals.

Ethanolic extract

This was achieved by the use of soxhlet ectractor in chemistry department laboratory of Kebbi State University of Science and Technology Aliero. 50.0 g of the powdered sample of (jatropha tanjorensis) was divide into two portions, then each portion were placed into a thimble and was then inserted into soxhlet. Each portion contain 50 g of the powder sample in the thimble was inserted into the soxhlet chamber, then the set up was assembled in which the soxhlet extractor chamber was placed into a pre weighed flask containing 500 cm^3 ethanol the pre weighed task was heated for about 45 min for each portion using heating mantle thermostat at 60°C temperature. After the extraction the solvent was recovered and the remaining solvent was distilled using water bath.

Water extract

The water extract of *jatropha tanjorensis* was prepared in the Laboratory by soaking 20 g of both samples in 100 ml of water and heat for 45 min. The mixture is then filtered and the filtrate kept and labeled for further analysis.

Percentage yield of extract

After distillation of the solvent, the beaker was placed on the electrical weight balance to take the reading

$$\text{Percentage yield} = \frac{W1 \times 100}{Wo}$$

Where Wo = percentage powdered sample in grams (g)
W1=percentage extraction in grams (g)

Phytochemical analysis

The extract was tested for the presence of secondary metabolites. Basic phytochemical analysis consist of performing chemical tests to detect the presence of alkaloids, flavonoids, glycosides, saponins, tannins, cardiac, glycosides, steroid, anthroquinone etc. (Harborne and Jefferey,1974).

Qualitative test for alkaloids

The oil extract (2 ml) was added to 2 ml of HCl to the acidic medium. 1 ml of dragendroffs reagent was added. An orange or red precipitate was immediately formed which indicate the presence of alkaloids.

Qualitative test for flavonoids

Three (3) drops of NH_3 solution were added in 1 ml of leaves extract, and then 0.5ml of concentrated HCl was added. A brown was observed indicating the presence of flavomoids.

Qualitative test for saponins

Saponins were qualitatively screened by the method of Harborne, (1998). 5 ml of the extract was placed in a test tube and 5 ml of distilled water added to the test tube and shaken thoroughly. The whole mixture was filtered.

Qualitative test for tannins

A small quality of the extract is boiled with 5ml of 45% solution of ethanol for 5 min. Each of the mixture is cooked and filtered. The different filtrates were used for the following test. 1 ml of the filtrate was diluted with distilled water and added with two drops of ferric chloride. A transparent greenish to black colour indicate the presence of tannins.

Qualitative test for steroids

2 ml of acetic anhydride was added to 0.5g of the extract with 2 ml of concentrated sulphuric acid. The colour change from violet to blue or green indicates a positive result for steroids.

Qualitative test for anthraquinones

5cm^3 of the plant extract was shaken with 10 cm^3 of benzene and 5cm^3 of 10% of ammonia solution was added. The mixture was shaken and the presence of a pink red colour in the ammonical (phase) indicates the presence of anthraquinones.

Qualitative test for volatile oils

2 ml of extract solution was shaken with 0.1m sodium hydroxide and a small quality of 0.1 m HCl. A white precipitate was formed with volatile.

Qualitative test for glycosides

5 ml of diluted sulphuric acid was added to the extract in a test tube and boiled for fifteen minutes in a water bath. It was then cooled and neutralized with 20% potassium

hydroxide solution. A mixture of 10ml of equal parts of feelings solution A and B were added and boiled for minute. A more dense red precipitate indicates the presence of glycosides.

Qualitative test for terpenoids

About 5 ml extract was mixed with 2 ml of chloroform and 2ml of conc. H_2SO_4 was added carefully added to form a layer. A reddish brown colouration of the interphase formed shows the presence to terpenoid (Soforowa, 1973).

Antimicrobial activity

Preparation of the extract for the antimicrobial activities

The media used in this research was prepared according to the manufactured instruction. (Antic diagonistic production in United Kingdom). The media are nutrient agar and nutrient both.

Nutrient agar

25 grams of the nutrient agar was weighed and transferred into a conical flask containing 1000 ml of distilled water it was then mixed. The mixture was heated to dissolve powder and it was then sterilized by autoclaving at 121°C for 15 min. It was then allowed to solidify.

Test for organisms

Pure strain of Escherichia Coli, Staphylococcus aureus and salmonella typhi were obtained from the microbiology laboratory, kebbi state university of science and technology Aliero. The isolates were checked for viability and purity before been transferred to nutrient agar slant and store. The inoculated plants were allowed to stand for 15mins before inoculating for 24 h at 37°C. Zone on inhibition around the wells indicated antibacterial activity against the bacterial. The diameter of the zone was measured diagonally.

Determination of zone inhibition

Fifteen millimeter (15 ml) of sterile nutrient agar was poured into each sterile petri dish of equal size and allowed to solidify. The surface of this sterile nutrient agar plate was streaked with the pure culture of standardized bacterial cell suspension. A cork borer (8mm in diameter) was sterilized by flaming and was used to create ditch at the centre of the plate. The hole so created was then filled the plant extract. The plate was allowed to stand for one hour for pre-difusion of the extract (Esimone *et al*., 1998) and incubated at 37°C for 24 h. At the end of the incubation period, the diameter of the zone of inhibition was measured in millimeter using meter rule (Ghamba, 2014).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The initial concentration of the plant extract (100 gm/l) was dilute double fold serial dilution by transferring 5 ml of the sterile plant extract (stock solution) into of sterile nutrient both to obtain 50 mg/ml of the concentration. The above process was repeated several times to obtain other dilution: 50 mg/ml,12.5mg/ml, 6.25mg/ml and finally 3.125 mg/ml (Ibekwe *et al*., 2001). Having obtained the different concentration of the next extract, each concentration was inoculated with 0.1ml of the standardized bacterial cell suspension and incubated at 37°C for 24 h. The growth of the inoculums in both is indicating by turbidity or cloudiness of both and the lowest concentration of the extract which inhibited the growth of the organism was taken as the minimum inhibitory concentration (MIC). Negative control was set up as follows: nutrient both only nutrient broth and sterile plant extract, and finally position control containing nutrient broth and test organism.

RESULTS AND DISCUSSION

The result of the phytochemical screening of the ethanolic and aqueous leaves and stem bark extract of jatropha tanjorensis are presented in (Table 1). The result of the antimicrobial activity of the leave and stem bark extract of *jatrogha tanjoresis* to some clinical pathogen using ethanolic and distilled water as solvents are shown in (Table 2). Phytochemical screening of the extracts is carried out to confirm the presence of some phytochemical constituents present in the sample. The leaves of *Jatropha tanjorensis* as well as the stem bark were screened for the presence of phytochemicals. On the leaves, glycosides, tannins, terpenoids and saponins were detected while volatile matter, reducing sugars and anthraquinones were absent as presented in (Table 1). Its use in traditional medicine can be attributed to the presence of these secondary metabolites which give the plant its characteristics medicinal value. The presence of tannins in a sample could be of important in its antimicrobial properties. Table 2 displays the result of the antimicrobial properties of the ethanolic leaves and stem bark extract with maximal activity recorded on S/typhy 7.00±0.000 by the leaves as against the standard drug

Table 1. The result of the phytochemical screening of the aqueous leaves and stem bark extract of *jatropha tanjorensis*.

Bioactive component	Leaves extract	Stem bark extract
ALKALOIDS	+	+
FLAVONOIDS	+	-
GLYCOSIDES	+	+
TANNINS	+	-
SAPONINS	+	+
ANTHRAQUINONES	-	-
REDUCING SUGAR	-	+
STEROIDS	+	-
VOLATILE	-	+
TERPENOIDS	+	-

KEY: += present and – not detected.

Table 2. Antibacterial activity of the aqueous and ethanolic leaves and stems bark of *jatrogha tanjoresis* on various strain of bacterial at various concentrations.

Extract cone (mg/ml)	S/A	S/typhi	Shigella	E.Coil	Strept. spp.
Ethanolic 100	5.40±0.424	7.00±0.000	4.15±0.122	5.10±0.14	3.90±0.141
Stem Bark 100	2.90±0.141	2.90±0.141	3.35±0.495	3.10±0.1	3.80±0.283
Ciproflaxicin 5ug/ml	22±0.08	12±0.02	10±0.02	21±0.03	15±0.058

KEY: S/A=Staphylococcus Aureus; S/typhi=salmonella typhi; S/Spp= streptococcus. Shows no zone of inhibition n=2

Table 3. The result of MIC antibacterial activity of aqueous and ethanolic leave extract of *jatropha tanjoresis* at various does on microbial strains.

Microorganism		*Jatropha tanjorensis*			Serial Dilution (ug/ml)		
Strep spp.	Ethanolic	+	+	+	+	+	+
Salm. Spp.	Ethanolic	+	+	+	+	+	+
E.Coil	Ethanolic	+	+	+	+	-	+
Shigella	Ethanolic	+	+	+	+	-	-

KEY: +=No growth (clear); -=growth (turbid); MIC=minimum inhibitory concentration

Table 4. The result of the MBC for antibacterial activity of aqueous and ethanolic leaves extract of *jatropha tanjornsis* at various doses on microbial strains.

Microorganism	*Jatropha tanjorensis*				Serial Dilution		
Strep spp.	+	+	-	-	-	-	-
Salm. Spp.	+	-	-	-	-	-	-
E.Coil	+	-	-	-	-	-	-
Shigella	-	-	-	-	-	-	-

KEY: +=No growth (clear); -=growth (turbid); MIC=minimum inhibitory concentration

which has 12±0.02. The result obtained reveals that the leaves extract are more effective than the stem bark extract. Similarly, of the two solvents used in the extraction, ethanol extract reveals higher activity against the test organisms. Also as shown, the (Tables 3 to 6) shows the result of the minimum inhibitory concentration (MIC) and minimum bacterial concentration at different doses. Table 3 displays little growth while Table 6 displays maximum growth which suggests that aqueous and ethanolic leaves and stem bark extract can be a potent antimicrobial agent and can therefore be employed in the treatment of microbial pathogenic

Table 5. The result of the MIC for antibacterial activity of aqueous and ethanolic stem bark extract of *jatropha tanjornsis* at various doses on microbial strains.

Microorganism	*Jatropha tanjorensis*			Serial dilution	
Strep spp.	+	+	+	+	-
Salm. Spp.	+	+	+	+	-
E.Coil	-	+	+	+	-
Shigella	-	-	-	-	-

KEY: +=No growth (clear); -=growth (turbid); MIC=minimum inhibitory concentration

Table 6. The result of the MBC for antibacterial of aqueous and ethanolic stem bark extract of *jatropha tanjornsis* at various doses on microbial strains.

Microorganism	*Jatropha tanjorensis*			Serial Dilution			
Strep spp.	+	-	-	-	-	-	-
Salm. Spp.	-	-	-	-	-	-	-
E.Coil	-	-	-	-	-	-	-
Shigella	-	-	-	-	-	-	-

KEY: +=No growth (clear); -=growth (turbid); MIC=minimum inhibitory concentration

diseases. These antimicrobial agents may act by inhibiting the nucleic acid, protein cell wall and membrane phospholipid biosynthesis to exert its effect (Franklin *et al.,* 1989).

Conclusion

The result of the findings showed that stem bark extract of *Jatropha tanjorensis* has antimicrobial properties against different strains of h human pathogenic bacteria. This activity can be attributed to the presence of certain phytochemical constituents present in the plant extract. The leaves were found to be more potent in activity at different concentrations. This therefore shows that the leaves of *Jatropha tanjorensis* can be used as an effective herbal medicine because of its high antimicrobial activity and its fewer side effects.

Authors' declaration

We declared that this study is an original research by our research team and we agree to publish it in the journal.

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