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Antimicrobial effects and Phytoconstituents of ethanolic extract of leaves of *Artemisia annua* L.

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Studies were carried out on antimicrobial effects and phytoconstituents of Ethanolic extract of leaves of *Artemisia annua* L. The extract significantly inhibited the test organisms. The minimum inhibitory concentration value of the ethanolic extract was 125 mg/ml for each of the test organisms: *Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas auroginosa*, *Klebsiella pneumoniae*, *Candida albicans* and *Staphylococcus aureus*. The result of the minimum bactericidal concentration showed that, the extract was bactericidal at the concentration of 250 mg/ml. The phytochemical analysis revealed the presence of Tannins, Flavonoids, Cardiac Glycosides, Alkaloids, Phenols and Resins.

Keyword: Antimicrobial, Phytoconstituents, *Artemisia annua* L., MIC, MBC.

1. Introduction

Artemisia annua L. belongs to the family, Asteraceae. It is known in the United States as sweet annie or annual wormwood. It is an annual herb, native to Asia, especially to China, where it is called ginghaosu. The plant has became naturalized in many countries including Romania, Hungary, Argentina, France, Italy, Bulgaria, the United States, Spain, and the former Yugoslavia [1,2,3,4]. Artemisia is mentioned in the Chinese Hand book of prescriptions for Emergency Treatments of 340 AD for treatment of fevers. In 1971, extraction of aerial parts of *Artemisia annua* with low-boiling solvents like diethylether, produced a compound mixture that had antimalarial properties on infected mice and monkeys. The main active principle, artemisinin (formerly referred to as arteannuin and as ginghaosu in chinese), was isolated and had its structure correctly defined in 1972 in China as a sesquiterpene lactone with an endoperoxide bridge. Artemisinin is now obtainable commercially in China and Vietnam as an antimalarial drug efficacious against drug-resistant strains of *Plasmodium malariae*, the malaria parasite. A semi synthetic drug based on artemisinin (artemether) has been recently registered in Africa as paluther. The Artemisinin also has toxic effect, even on *Artemisia annua*, and is a candidate for natural herbicide [5,6].

Artemisinin production by *Artemisia annua* is usually in the range of 0.01% to 0.4% but some clones produce over 1% [7]. Artemisinin can also be gotten from Artemisinic acid which occurs at concentration as much as 10 fold higher than artemisinin [8]. Vonwiller and colleagues [9] reported an extraction method which makes possible the extraction of both compound from the same plant material, hence increasing the final production of artemisinin. It is also effective in the treatment of cerebral malaria. Likewise its effectiveness has been demonstrated in the treatment of skin disease. This study is aimed at determining the antimicrobial effects and

phytoconstituents of ethanolic extract of leaves of Artemisia annua L.

2. Materials and Methods

2.1 Collection of Plant

The samples of *Artemisia annua* at near flowering stage were collected from the nursery farm of centre for Biotechnology and Genetic Engineering located at Gangnin in Langtang south Local Government Area of Plateau State.

2.2 Drying of Plant Material

The plants samples were allowed to dry at room temperature. The dried leaves were then pulverized with the aid of sterilized mortar and pestle

2.3 Extraction

Extraction from the dried sample was carried out using soxhlet extractor. A weight of 40 gm of the powdered sample was subjected to exhaustive soxhlet extraction in ethanol at 70 °C for 72 hour. The resultant solvent was then evaporated using water bath maintained at 100 °C. The resultant extract was then stored in the refrigerator until ready for use.

2.4 Phytochemical Analysis

A number of tests were conducted on the ethanolic extract obtained from *A annua*. Standard methods were used for each of these tests which included test for the presences of saponins, tannins, anthraquinone, flavonoids, steroid, alkaloid, terpenes, cardiac glycoside, phenols and resins.

2.4.1 Test for Saponins

A weight of 2 gm of the extract was boiled in 20 ml distilled water with the aid of a water bath and the resultant mixture was filtered using a filter paper. A volume of 10 ml of the filtrate was mixed with 5 ml distilled water and shaken vigorously in a test tube. A stable persistent froth was taken as the preliminary evidence for the presence of saponins [10].

2.4.2 Test for Tannins.

A weight of 5 gm of the extract was stirred with 10ml distilled water, then filtered, ferric chloride reagent was then added to the filtrate. The blue-black, green or blue-green precipitate was taken as an evidence for the presence of tannins [11].

2.4.3 Test for Alkaloid

A weight of 0.5 gm of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid in a conical flask placed in a water bath. A volume of 1ml of the filtrate was then mixed with drops of Mayer's reagent and a second 1 ml portion was treated with 2 drops of Dragendroff's reagent. Turbidity or precipitation with either of these reagents was taken as the preliminary evidence for the presence of alkaloid

2.4.4 Test for Anthraquinones

Borntrager's test was used as described by Evans [11] for the detection of anthraquinone. 5 gm of the extract solution was shaken with 10ml benzene, filtered and 5 ml of 10 percent ammonia added to the filtrate. The mixture was shaken and the presences of a pink red in the ammonia (lower) phase indicated the presence of anthraquinone.

2.4.5 Test for Cardiac Glycosides

(Legal test: test for cardenolide aglycone)

A weight of 2 gm of the extract was dissolved in pryridine and 2 drops of 2% Sodium nitroprusside together with the 2 drops of 20% Sodium hydroxide were added. A deep red colour which faded to a brownish yellow indicated the presence of cardenolides.

2.4.6 Test for Flavonoids

The method employed was that described by Trease & Evans [12]. A weight of 5 gm of the plants leaf extract was added to 5 ml of diluted ammonia solution, followed by addition of concentrated sulphuric acid. A yellow colour indicated the presence of flavonoids.

2.4.7 Test for Resins

A weight of 0.5 gm of the leaf extract was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid was then added. A

purple or violet colour indicated the presence of resins.

2.5 Preparation of Test Organisms

The test organisms used were pure culture of *Escherichia coli, Streptococcus faecalis, Candida albican, Pseudomonas auroginosa, Staphylococcus aureus,* and *Klebsiella pneumoniae.* These organisms were obtained from the Central Diagnostic Laboratory Department of the Federal Medical Centre, Keffi. The said test organisms were inoculated onto nutrient broth for a period 24 hour and at growth temperature of 37 °C in order to ascertain their viability.

2.6 Susceptibility Test of organisms by Agar Well Diffusion Method

Plates of nutrient agar media were prepared. The plates were inoculated with the test organisms in duplicates by spread plate method. A cork borer of width 6 mm in diameter was flamed using a Bunsen burner and was used to bore wells on the inoculated plates. The wells were then filled with the extracts at different concentrations, that is 500, 250, 125 and 62.5 mg/ml. Standard 4mg/ml Gentamycin was used as control. The extracts were allowed for 1 hour to diffuse into the agar plates and then incubated at 37 °C for 24 hours. The potency of the extracts was determined by the clear zones of inhibition around the wells and these respectively measures at nearest millimeter as diameter zones of inhibition.

2.7 Minimum Inhibitory Concentration

A weight of 0.25 gm (250 mg) of extract was dissolved in 4 ml nutrient broth. Subsequently, two folds serial dilutions were made from the original stock of 4ml containing 250 mg to obtain the following concentrations, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0.98 Mg/ml^{-1} . Standardized inoculums of each test organism was introduced into the mixture and incubated at 37 °C for 24 hours. The lowest concentration of the extract that inhibited the test organisms was considered as the minimum inhibitory concentration (MIC).

2.8 Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) was determined by the method of Nester and colleagues [13]. All minimum inhibitory concentration (MIC) tubes that showed no microbial growth after 24 hours of incubation were sub cultured by streaking onto the surface of freshly prepared nutrient agar plates and incubated at 37 °C for 24 hours. The MBC was taken as the least concentration of the extract that did not allow any visible bacterial growth on the nutrient agar plate.

3. Result

Results of Phytoconstituents analysis of *Artemisia annua* are shown in Table 1.

Table 1: Phytochemical Screening of *Artemisia Annua*

Phytochemical compounds	Ethanolic extract
Tannins	+
Flavonoids	+
Cardiac glycosides	+
Alkaloids	+
Phenols	+
Resins	+
Saponins	-
Anthraquinones	-
Steroids	-
Terpenes	-

+: Present,
- : Absent

The result revealed that Tannins, Flavonoids, cardiac glycosides, alkaloids, phenols and resins were the bioactive component found in ethanolic extract of *Artemisia annua* while saponins, anthraquinones steroid, and Terpenes were absent in ethanolic extract of *Artemisia annua.*

Table 2: Effect of Various Dilution of Ethanolic Extract of *Artemisia Annua*

Test organism	Extract concentration/Zones of inhibition (mm)				Gentamycin
	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	4mg/ml
Escherichia coli	22.00	18.00	16.00	10.00	26.00
Streptococcus faecalis	28.50	24.00	22.00	18.00	35.50
Candida albican	16.00	14.00	12.50	10.00	20.00
Pseudomonas auroginosa	20.00	18.50	20.00	18.50	30.50
Klebsiella pneumoniae	16.00	12.50	10.00	10.00	40.50
Staphylococcus aureus	21.00	20.00	16.00	12.00	20.50

Table 3: Determination of MIC's (mg/ml) of Ethanolic Extract of *Artemisia annua*

Test Organisms	Extraction Concentration									
	250	125	62.5	31.25	15.63	7.81	3.91	1.95	0.98	control
E. coli	-	-	+	+	+	+	+	+	+	125
S. faecalis	-	-	+	+	+	+	+	+	+	125
Candida albicans	-	-	+	+	+	+	+	+	+	125
P. auroginosa	-	-	+	+	+	+	+	+	+	125
K. pneumoniae	-	-	+	+	+	+	+	+	+	125
S. aureus	-	-	+	+	+	+	+	+	+	125

\- = No Growth
\+ = Growth

Table 4: Determination of MBC of Ethanolic Extract

Test Organisms	Extract Concentration		
	250mg/ml	125mg/ml	MBC
Escherichia coli	-	-	250
Streptococcus faecalis	-	-	250
Candida albicans	-	-	250
Pseudomonas auroginosa	-	-	250
Klebsiella pneumoniae	-	-	250
Staphylococcus aureus	-	-	250

4. Discussion

The antimicrobial activity of *Artemisia annua* was determined with the view to know the degree of efficacy and potency of ethanolic extract of leaves of *Artemisia annua* for the treatment of microbial infection.

The ethanolic extract of the leaves of Artemisia annua used revealed the bioactive compound from the phytoconstituents analysis to include tannins, flavonoids, cardiac glycosides, Alkaloids phenols and resins. The ethanolic extract of leaves of Artemisia annua inhibited the growth of *Staphylococcus aureus, Klebsiella pneumoniea, Candida albicans, Escherichia coli, Pseudomonas auroginosa and Streptococcus faecalis.* The inhibitory effect was realized to be as a result of the bioactive component found in the leaves extract of *Artemisia annua* L.

The results showed that, at 125 mg/ml, 250 mg/ml concentrations of the ethanolic extract, all test organisms were inhibited. The minimum inhibitory concentration of the ethanolic extract was determined. The result showed that the MIC of ethanolic extract was 125 mg/ml for all the organisms tested. The results are shown in table 3. The minimum bactericidal concentration of the ethanolic extract was determined and the result showed that at the concentration of 125 mg/ml,

250 mg/ml there was no growth after sub culturing onto nutrient agar plate. This therefore mean that the extract was bactericidal that the concentration of 250 mg/ml.

The result of the minimum inhibitory concentration in this investigation showed that, the plants used for this research work has high bactericidal effect against almost all the clinical isolates of the test organisms used. Therefore, from our findings, it was observed that, the plant extracts used for this research work can be duly used for the treatment of some ailments caused by the test organisms.

5. Conclusion

The finding revealed that, *Artemisia annua* contain bioactive compound that are effective against the test organisms thus the crude extracts could be used directly for chemotherapy or treatment of infections caused by these organisms. The ethanolic extract of *Artemisia annua* showed zones of inhibition against the test organisms used for this research work. The result obtained from the Phytoconstituent and antimicrobial tests of this plant extracts revealed that the bioactive component, were found to be active against the microorganisms. Therefore there is a need for further investigation in order to isolate the specific bioactive compounds. The pharmaceutical industries are advised to take advantage of such kind of investigation and then invest further studies, which could lead to the isolation, identification and characterization of the specific bioactive compounds found in this plant. This would also lead to a more efficient production of antimicrobial drugs that originates from this plant as they could be very cheap and affordable.

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