Available online at www.jpbms.info

Research article

ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Screening ethanolic and aqueous leaf extracts of *Taraxacum offinale* for *in vitro* bacteria growth inhibition

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Abstract:

The increased incidence of bacteria resistance to many antibacterial drugs is of great concern and medicinal plants have proven as an alternative source of antibacterial agents. *Taraxacum officinale* is a stemless herb used as food vegetable and medicine. The plant is found chiefly as a persistent weed in many temperate regions. Previous reports show that the leaves of *T. officinale* contain bioactive components. In the current research, the ethanolic and aqueous leaf extracts of *T. officinale* were screened for phytochemicals and *in vitro* antibacterial activity. *Escherichia coli*, *Klebsiella pneumoneae*, *Pseudomonas auregenosa* and *Staphylococcus aureus* were used as test organisms. Data was analyzed using Mann-Whitney U-test and P values less than 0.05 were considered significant. Preliminary phytochemical screening of aqueous and ethanolic leaf extract of *T. officinale* revealed the presence of saponins, phenolics, triterpenes tannins, phytosteroids and reducing sugar some of which have been reported to be bioactive. The bacterial growth inhibition activity of the extracts was evaluated using agar well diffusion method at concentrations ranging from 50mg/ml to 200mg/ml. At concentration 200mg/ml, the ethanolic leaf extract showed inhibition against only *E. coli* and *S. aureus* with mean diameter of zone of inhibition of 23.50±1.00 mm and 10.75±1.50 mm respectively. At concentration 100mg/ml, the mean diameter zone of inhibition for the ethanolic extract were 16.00±2.83 mm and 9.00±0.82 mm against *E. coli* and *S. aureus* respectively. At concentration 50mg/ml, the ethanolic leaf extract showed inhibition against only *E. coli* with mean diameter of the zone of inhibition of 10.50±1.00 mm. The aqueous extract on the other hand showed inhibition against *E. coli* only at 200mg/ml and 100mg/ml. The result reveals that antibacterial activities of aqueous and ethanolic leaf extract of *T. officinale* were concentration dependent. The result further suggests that the ethanolic extract of *T. officinale* was the most active compared with the aqueous extract. Of the bacteria tested, *E. coli* was the most susceptible to the extracts. In conclusion, both aqueous and ethanolic extract of *T. officinale* possess significant ($P < 0.05$) antibacterial activity and may be very useful in the discovery of novel antibacterial agents.

Keywords: Antibacterial assay, *Taraxacum officinale*, agar well diffusion, Phytocompounds

Introduction:

Plants have been used widely as source of medicine by people of all cultures for treating different ailments. Traditional medicine is an important part of the health care system in most African countries. About 80-90% of the people refer to herbs and traditional healers for primary health care [1]. Plant medicine is still used by most of the world's population, mainly in developing countries for primary health care partly because of better cultural acceptability, better compatibility with human body and fewer side effects as compared to orthodox drugs.

The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide [2]. Many investigations are currently conducted on medicinal plants on the basis of information supplied by the local populations with the object of finding out phytochemical constituents for application in the prevention and treatment of infectious diseases and other diseases of non-microbial etiology. Several researches have been conducted to provide scientific basis for the efficacy of plants in herbal medicines. Investigations based on ethno-pharmacological information are generally considered an effective approach in the discovery of new anti-infective agents from higher plants.

Several scientific reports have shown that medicinal plants contain active phytocompounds. These substances are potentially significant in therapeutic applications against human and animal pathogens, including bacteria, fungi, and viruses [3].

Extraction and use of synthetic as well as inorganic antibiotics are expensive and pose serious threat to health and environment. Apart from this, synthetic drugs are expensive and not readily available to most communities in the rural areas and this has shifted attention to the use of traditional herbal drugs.

The global need for alternative prevention and treatment option and product for infectious diseases arises from increase in disease incidence, increase resistance by pathogenic bacteria and financial consideration in the developing world. Quite a number of drugs obtained from medicinal plants are commercially used for treatment of one ailment or the other. In spite of the advances made in the discovery of bioactive principles from plants, the

medicinal potentials of many plants still remains undiscovered. Thus, scientists are in a constant search for bioactive substances of plant origin. The continued investigation of plant secondary metabolites has led to important breakthroughs in pharmacotherapeutics in Africa and other parts of the developing world [4]. These researches have suggested that traditional medicine seems to have certain advantages over the orthodox system of medicine because it forms an integral part of the people's culture and it is particularly effective in solving certain cultural health problems [5].

The plant *T. officinale* belongs to the family Asteraceae. It grows to a height of about 12 inches; traditionally *T. officinale* is commonly used as food. The leaves are used in salads and tea while the roots are often used as a coffee substitute. *T. officinale* leaves and roots extracts have been used for hundreds of years to treat liver, gallbladder, kidney, and joint problems [6]. In some communities, *T. officinale* is considered a blood purifier and is used for ailments as varied as eczema and cancer. As is the case today, *T. officinale* has also been used historically to treat poor digestion, water retention, and diseases of the liver, including hepatitis [7]. It has also been used in ancient Chinese culture to cure bacterial infections and ailments. In Ghana however, *T. officinale* is often regarded as weed and has seen very little attention in the scientific community. To the best of our knowledge, the antimicrobial potential of *T. officinale* has not been explored extensively. This research seeks to evaluate the aqueous and ethanolic extract of *T. officinale* for antibacterial activity. The phytochemical profile of the extracts will be determined qualitatively and the antibacterial potential evaluated *in vitro* using *E. coli, K. pneumonia, S. aureus and P. auregenosa* as test organisms.

Material and Method:

Chemicals:

Most of the chemicals used in present study were obtain from (BDH international Ltd, BH15 1TD, England). Potassium iodate (analytical grade) was obtained from Panraec, Spain.

Equipment and apparatus:

The equipment and apparatus used in the project were incubator (JP Selecta, Buch and Holm A/S), water bath, Electronic balance (Sartorius SP224S), Autoclave (JP Selecta, S.A N-11 Km 585, Barcelona, Spain), magnetic stirrers (RCT basic, WERKE).

Plant material and microorganisms:

The materials used include leaves of *T. officinale*, Mueller Hinton agar (Oxide Ltd, Basingstoke, and Hampshire, England), *E. coli, S. aureus, K. pneumoneae and P. aureginosa.*

Methodology:

Plant collection and treatment:

Fresh leaves of *Taraxacum officinale* were harvested randomly at Ejura in Ashanti region of Ghana on 14th march, 2012 and was verified and authenticated by Mr. Appiah Kubi a lecturer in the Department of Applied biology of the University for Development Studies, Ghana. Adequate amount of the leaves of the plant were sundried for 24 hours and then milled into powder.

Preparation of T. officinale aqueous leaves extracts:

About 500g of the powdered sample of *T. officinale* was weighed on the electronic balance in to a container. Approximately 2 liters of distilled water was added, the mixture was boiled for 45minutes and the solution of the extract allowed to cool and then transferred in to a flask.

Preparation of *Taraxacum officinale* ethanolic leaves extracts

About 300g gram of the powdered sample of the *T. officinale* was weighed on an electronic balance into a container, 1.5liters of 70% ethanol was added, the container was covered for about 24 hours and the extraction was done using wise guess. The extract was concentrated using water bath.

Phytochemical screening:

Several phytochemical tests were carried out to detect the presence of phytochemical components in both ethanolic and aqueous extract of *T. officinale* using standard procedure. The standard procedures used in this research adopted from the works of Sofowora [8], Harborne [9] and Evans [10].

Antimicrobial assay:

Standard methods were adopted in antibacterial assay. The microorganisms used in the study were *E. coli* and *K. pneumonia, S. aereus* and *P. auregenosa*. These microbes were of standard strains purchased at the Tamale teaching hospital. The microbes were cultured on a peptone agar and incubated at 37°C for 16 hours. The microbes were sub-cultured and incubated for 2 hours to obtain pure colonies. Mueller-Hinton was used in an agar well diffusion method for all the bacteria.

In preparing the agar for culturing the bacteria, 27g of Mueller-Hinton agar was dissolved in 720ml of distilled water and the mixture was stirred to form a clear solution to ensure that it was well mixed. The solution was then kept in an autoclave at the temperature of 121°C for 45minutes to ensure maximum sterility. Each microbe was sub cultured in to a 5ml of peptone water and was incubated for about 2hours to serve as broth.

The sterilized media solution was allowed to cool to a temperature of about 60°C. 20ml of the media solution were then transferred in to sterilized and well labeled media plates used for the media and then incubated for 24hours to determine possible media contamination. The sub cultured microbes were then inoculated in to the media in the plates using micro pipette and were then allowed to spread properly. A sterile cork borer (5mm in diameter) was used to make wells on the media in the plates.

The concentration of aqueous solutions made from the extracts were varied as follows; 200mg/ml, 100mg/ml, and 50mg/ml

Chloramphenicol a product of Letap Pharmaceutical Company in Ghana manufactured on 17th December 2011 with expiry date of December, 2013 was used as positive control. About 100mg of the Chloramphenicol was dissolved in 10ml of distilled water to prepare a 10mg/ml solution.

About 100µm of each extract's solution and standard (Chloramphenicol) were introduced in to each hole of different plate in triplicate. The plates were left for the extracts to diffuse into the media before it was placed in the incubator at 37°C for 24 hours. The relative

susceptibility of the organism to the extract is indicated by clear zone of inhibition produced after incubation which was measured and recorded in millimeters.

Statistics:

All data were recorded on standardized forms. Data expressed as the mean ± standard error of the mean (n = 3) and significance was determined using Mann-Whitney U-test. The mean values were considered significantly different if $p < 0.05$.

Results and Discussion:

Phytochemical analysis:

The screening of phytochemical constituents in *T. officinale* revealed the presence of saponins, phenolics, reducing sugar, anthracenosides, triterpenes, steroids, tannins and phlobatanins in both ethanolic and aqueous extract. This is presented in ***table 1***. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *T. officinale.* Also, these classes of compounds are reported to have therapeutic activity against several pathogens and therefore the result supports the use of *T. officinale* traditionally for the treatment of various illnesses. These secondary metabolites show antimicrobial activity through different mechanisms. Saponins have been reported to be useful in managing inflammation [11]. According to Parekh and Chanda [12], tannins react with proteins to provide tanning effect which is important for the treatment inflammations. Steroidal extract on the other hand have medicinal properties in plants and has been worded by Sudha and Vinodhini [13].

Table 1. Phytochemical constituents of aqueous and ethanolic leaf extract of T. officinale

Phytochemicals	Aqueous extract	Ethanolic extract
Alkaloids	Negative	Negative
Saponins	Positive	Positive
Reducing sugar	Positive	Positive
Phenolics	Positive	Positive
Anthracenosides	Positive	Positive
Triterpenes	Positive	Positive
Flavonoids	Negative	Negative
Phytosteroids	Positive	Positive
Tanins	Positive	Positive
Phytobatanins	Negative	Negative

Positive → presence of phytochemical
Negative → absence of phytochemical

Antibacterial activity assay:

Both extracts showed varied degree of antibacterial activity against the organisms used. The result is presented in ***table 2***. Using the Mann-Whitney Test, the activity of the extracts was statistically significant ($P < 0.05$) compared with the negative control. The ethanolic extract of *T. officinale* showed inhibition of growth of *S. aureus* and *E. coli*, but no zones of inhibition were shown against *P. auregenosa* and *K. pneumoniae.* At concentration 200mg/ml, the mean diameter of zone of inhibition against *E. coli* and *S. aureus* were 23.50±1.00 mm and 10.75±1.50 mm respectively. Also at concentration 100mg/ml, the mean diameter of zone of inhibition against *E. coli* was 16.00±2.83 mm and that of *S. aureus* was 9.00±0.82 mm.

At concentration 50mg/ml, the ethanolic leaf extract showed zone of inhibition only against *E. coli* with mean diameter of 10.50±1.00 mm. This result is consistent with previous reports [14]. The result further suggests that minimum inhibition concentration of the ethanolic leaf extract against *E. coli* was below 50mg/ml. *E. coli* was more susceptible to the ethanolic leaf extract when compared with *S. aureus*. The extent of growth inhibition was concentration dependent.

All the bacteria tested demonstrated resistance against the aqueous extract of *T. officinale* at all concentrations with the exception of *E. coli* which was susceptible only at 200mg/ml. Although the extracts contain similar phytochemicals, the result suggests that the active constituents may be more soluble in ethanol than in water as manifested in the antibacterial activities of both extracts. The antibacterial activity of the aqueous extract is in agreement with results from previous reports [15] which suggest a low antibacterial activity of the aqueous leaf extract.

The presence of different phytochemical constituents in the leaf extract is offering the therapeutic basis for the antibacterial activities observe in these extracts.

Table 2a. Mean diameter of zone of inhibition of ethanolic leaf extract of *T. officinale.*

Organisms	Mean diameter of the zone of inhibition [Mean±SEM (mm)]			Control (Chloramphenicol)
	200mg/ml	100mg/ml	50mg/ml	
E. coli	23.50±1.00	16.00±2.83	10.50±1.00	30.50±0.71
S. Aureus	10.75±1.50	9.00±0.82	0.00±0.00	25.00±1.41
K. pneumonia	0.00±0.00	0.00±0.00	0.00±0.00	26.50±0.71
P. auregenosa	0.00±0.00	0.00±0.00	0.00±0.00	26.00±0.00

Values are expressed as mean±SEM (n = 3) and analyzed statistically using the Mann-Whitney Test $\alpha = 0.05$

Table 2b. Mean diameter zone of inhibition of aqueous leaf extract of *T. officinale*

Organisms	Mean diameter of the zone of inhibition [Mean±SEM (mm)]			Control (chloramphenicol)
	200mg/ml	100mg/ml	50mg/ml	
E. coli	7.50±1.00	5.25±2.83	0.00±0.00	30.50±0.71
S. Aureus	0.00±0.00	0.00±0.00	0.00±0.00	25.00±1.41
K. pneumonia	0.00±0.00	0.00±0.00	0.00±0.00	26.50±0.71
P. auregenosa	0.00±0.00	0.00±0.00	0.00±0.00	26.00±0.00

Values are expressed as mean±SEM (n = 3) and analyzed statistically using the Mann-Whitney Test $\alpha = 0.05$

Conclusion:

The current research has shown *in vitro* activity of the aqueous and ethanolic leaf extracts of *T. officinale* as antibacterial agent against *E. coli*, *K. pneumoniae*, *P. auregenosa* and *S. aureus*. From the result, ethanolic extract of *T. officinale* was active against *E. coli* and *S. aureus*. The result further suggests that the ethanolic extract of *T. officinale* possess greater antibacterial activity compared with the aqueous extract. In conclusion, both aqueous and ethanolic extract of *T. officinale* possess antibacterial properties and could be very useful in our quest to search for novel antibacterial agents.

Acknowledgement:

The authors are thankful to the Departments of Applied Biology and Applied chemistry & Biochemistry of the University For Development Studies, Navrongo, Ghana for providing facilities for this project. The authors are also thankful to all authors whose works have been cited in this paper for the diverse literature that they have provided in the preparation of the manuscript.

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