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Data in Journal of Applied Pharmaceutical Science · April 2014

DOI: 10.7324/JAPS.2014.40309

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# An *in vitro* analysis of antiproliferative and antimicrobial activities of solvent fractions of *Taraxacum officinale* (Dandelion) leaf

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## ARTICLE INFO

### Article history:

Received on: 21/11/2013

Revised on: 08/12/2013

Accepted on: 03/01/2014

Available online: 30/03/2014

### Key words:

Antiproliferative, antimicrobial, solvent fractions, *Taraxacum officinale* leaf extract.

## ABSTRACT

The persistent upsurge in multidrug resistance of common pathogens and global increase in the incidence of non-infectious diseases such as cancers and diabetes has led to an urgent need for the discovery of novel and affordable pharmaceuticals. *Taraxacum officinale* commonly called dandelion has been used in traditional medicine for the treatment of various conditions. Although the antimicrobial and antiproliferative properties of various crude extracts of *T. officinale* have been reported, there is little report on the antimicrobial and antiproliferative activities of its solvent fractions. In this study, various solvent fractions from the crude methanolic extract of *T. officinale* leaves were examined for their antiproliferative and antimicrobial activities with the aim of providing a baseline data for the discovery of the antiproliferative and antimicrobial phytochemicals in *T. officinale* leaves. Whereas, methylene chloride fraction showed the highest antiproliferative activity, inhibiting 97 % proliferation of SGT oral cancer cells the butanol fraction showed the highest antimicrobial activity in both disc diffusion and broth dilution assays. Therefore the methylene chloride and the butanolic fractions could respectively act as leads for the discovery of important anticancer and antimicrobial phytochemicals from *T. officinale*.

## INTRODUCTION

The need for the search for novel and affordable pharmaceutical agents is now more urgent than ever. This is not only because of the upsurge in emergence of multidrug resistance in common pathogens (WHO, 2000) but also the global increase in the incidence of many non-infectious diseases such as cancer (Jemel *et al.*, 2011), diabetes (Wild *et al.*, 2004) and cardiovascular diseases. In the late 1960s and early 1970s, antimicrobial drugs have notably chalked a remarkable success leading to a general believe that infectious diseases had been conquered. However, 40 years down the line, infectious diseases remain the second-leading cause of death worldwide (Spellberg *et al.*, 2004) mainly because of the emergence of multidrug resistance in many pathogens (Howard *et al.*, 2003; Spellberg *et al.*, 2004; WHO, 2000). In addition, many non-infectious diseases such as cancer, diabetes and cardiovascular disorders which were once thought to be diseases of the affluent and developed nations have become global (Hosain *et al.*, 2007; Jemel *et al.*, 2011; Wild *et al.*, 2004) and the incidence of cancer in developing countries

for instance is projected to surpass that of the developed countries by 2020 (Kanavos, 2006). Unfortunately, the development of new pharmacological agents is not keeping pace with the global upsurge of multidrug resistance and incidence of non-infectious diseases and the process of development of these new pharmacological agents seemed to be facing significant obstacles (Spellberg *et al.*, 2004). The situation is even more alarming in developing countries where expertise in the area is lacking and research and development is lagging behind (Sofowora, 1992). Nonetheless, plants still offer an alternative source of valuable medicine for both developing and developed countries (Rates, 2001). Especially in cancer chemotherapy, natural products have contributed overwhelmingly to the expansion of the chemotherapeutic arsenal in that 50% of all the anticancer drugs approved worldwide between 1940 and 2006 were either natural products or natural product derived (Newmann and Cragg, 2007). *Taraxacum officinale* commonly called “dandelion” in English has long been used in herbal medicine for its choleric, antirheumatic and diuretic properties (Petlevski *et al.*, 2001; Sweeney *et al.*, 2005). Although the antimicrobial (Ghaima *et al.*, 2013) and antiproliferative properties (Menghini *et al.*, 2010; Sigstedt *et al.*, 2008; Sweeney *et al.*, 2005) of various crude extracts

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of *T. officinale* have been reported, there is little report on the antimicrobial and antiproliferative activities of its solvent fractions. Furthermore, the ever increasing evidence associating plant extracts with high risk of health complications (Peterson *et al.*, 2012) has made the call for isolation and reformulation of active phyto-compounds from a mixture of potentially toxic constituents stronger in recent times. The present study therefore investigated potential anticancer and antimicrobial activities of the various solvent fractions of dried dandelion leaves with the aim of providing a baseline data for identifying the active anticancer and antimicrobial components.

The methylene chloride fraction showed the highest antiproliferative activity, inhibiting 97 % proliferation of SGT oral cancer cells at 200µg/ml. This was followed by the ethyl acetate (42.03 %) and butanol (24.35 %) fractions. Moreover, the extracts were nontoxic to normal cells in the LDH assay. The butanol fraction showed the highest antimicrobial activity in both the disc diffusion and broth dilution assays. Therefore the methylene chloride and the butanolic fractions leaves could act as leads to the discovery of important anticancer and antimicrobial phyto-compounds respectively from *T. officinale*.

## MATERIALS AND METHODS

### Preparation of plant material and solvent fractionation

Fresh *T. officinale* leaves were collected and washed in distilled water, air dried for a month and ground to fine powder. Crude methanolic extract was prepared from 1.50 kg of the dried powdered leaves in 2 L of aqueous methanol (70 % v/v), evaporated and then freeze dried. Hexane, methylene chloride, ethyl acetate and butanol fractions were obtained by sequentially fractionating aqueous mixture of 205 g of the freeze dried sample with hexane, methylene chloride, ethyl acetate and butanol. Each solvent fractionation was repeated twice, pooled and freeze dried.

### Estimation of total phenolic and flavonoid contents

The total phenolic content in both crude extract and fractions was measured as described by Chang *et al.*, (2002). Exactly 0.5 g of each sample was dissolved in 5.0 ml methanol and added to 0.5 ml Folin–Ciocalteu reagent for 5 min. Sodium carbonate (1 ml of 1N) was added and incubated for 1 h at room temperature. The absorbance was measured at 765 nm. Calibration curve was prepared using 20, 40, 60, 80 and 100 mg/ml solutions of gallic acid in methanol. Total phenolic contents were calculated as milligrams of gallic acid per gram dry weight of sample or gallic acid equivalent (GAE).

The flavonoid content in both crude extract and fractions was measured as described previously (Singleton *et al.*, 1999). Exactly 0.5 g of each sample was dissolved in 5.0 ml of methanol and added to 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.0 ml distilled water. The reaction mixture was incubated at room temperature for 30 min and the absorbance measured at 420 nm. Calibration curve was prepared using 20, 40, 60, 80 and 100 mg/ml concentrations of quercetin.

Total flavonoid content was presented as milligrams of quercetin per gram dry weight of sample.

### Determination of antiproliferative activity of the extracts on SGT oral cancer cells and RAW 264.7 macrophage cells

The antiproliferative effects of the solvent fractions on SGT oral cancer cells and RAW 264.7 macrophage cells were evaluated using the 3,4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide (MTT) assay as described previously (Mosmann, 1983). The cells were grown in 24 wells at  $2.5 \times 10^5$  cells/ml for 24 h in a 5% CO<sub>2</sub> and 95% humidified atmosphere at 37 °C. To 500 µl of cell culture, 500 µl of 0.2 g/ml of the extracts or curcumin (positive control) dissolved in 5 % Dimethyl sulfoxide (DMSO) was added and incubated at 37 °C for 48 h. The supernatant was decanted and 250 µl of MTT (5 mg/ml) added to each well and incubated at 37 °C for 4 h. The supernatant was again discarded and the formazan crystals formed were dissolved in DMSO and absorbance read using an ELIZA microplate reader at 590 nm. Negative control samples were made by replacing plant extracts with equal volumes of 5% DMSO.

### Evaluation of cytotoxic effects of the extracts on RAW 267.4 macrophage cells

The cytotoxic effect of the extracts was examined on RAW 264.7 macrophage cells using the method by Patel *et al.*, (2009). Cells were seeded at  $2 \times 10^5$  cells /ml in 24 wells. A 500 µl of 400 µg/ml of each fraction dissolved in DMSO was added to 500 µl of cell suspension and incubated at 37 °C for 24 h. The supernatant was collected and the cells were lysed with cell lysis solution at room temperature. LDH activity was measured in both supernatant and lysate. A mixture of 100 µl malast reagent and 25 µl NBT/PMS was placed in duplicates into a 96 well microtitre plate. To the malast-NBT/PES mixture, 20 µl of supernatant or lysate was added and incubated for 5 min. The plate was then placed into a microplate reader set at a wavelength of 620 nm and readings recorded. Cytotoxicity was calculated as:

$$\text{OD}_{(\text{supernatant})} / \text{OD}_{(\text{supernatant and lysate})} \times 100.$$

### Evaluation of antimicrobial activity

#### Disc diffusion method

This was carried out as described by Karou *et al.*, (2006) against *E. coli*, *S. aureus*, *B. subtilis*, *C. albicans* and *S. cerevisiae*. Organisms were grown overnight at 37 °C, after which 500 µl aliquots of each culture ( $1 \times 10^6$  CFU/ml) was spread on Mueller Hinton agar. After 10 minutes of drying, paper discs loaded with 70 µl of different concentrations of extracts and reference drug all dissolved in DMSO were placed on the surface of cultured plates and incubated at 37 °C for 24 h. The diameters of inhibition zones were measured. DMSO was used as negative control. Tetracycline (100 µg/ml) was used as a positive control.

#### Broth dilution method

Percentage inhibition of microbial growth and minimum inhibitory concentrations of the extracts were evaluated against the

test microbes as described previously (Wiegand *et al.*, 2008) with minor modifications. Microbes were grown overnight at 37 °C in nutrient broth and optical density adjusted to  $1 \times 10^6$  CFU/ml. To 9.5 ml of microbial suspension in test tubes, 500  $\mu$ l of extracts dissolved in DMSO were added to make a final concentration of 2.5 mg/ml and incubated at 37 °C for 24 h. The content of each test tube (200  $\mu$ l) was transferred into 96 well plates and incubated with 50  $\mu$ l of 5 mg/ml 2,3,5-triphenyltetrazolium chloride for 2 h. Absorbance was measured at 570 nm and percentage inhibition of cell growth was evaluated. Negative controls were made by replacing extracts with DMSO. For vehicular controls, nutrient broth was used in place of microbial suspension. Experiments were performed three times.

### STATISTICAL ANALYSIS

Values were expressed as means  $\pm$  standard error mean (SEM) of 3 independent experiments. Comparisons between means were performed and significance was evaluated by analysis of variance (ANOVA) followed by Duncan's protected least significant difference test. Probability value of  $p < 0.05$  was used as the criteria for significant differences.

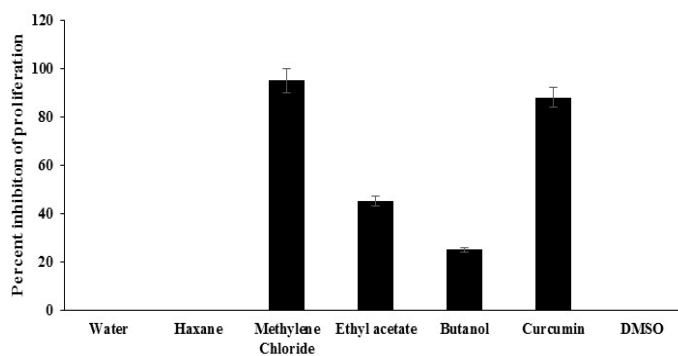
### RESULTS AND DISCUSSION

Although the antimicrobial (Ghaima *et al.*, 2013) and antiproliferative properties (Menghini *et al.*, 2010; Sigstedt *et al.*, 2008; Sweeney *et al.*, 2005) of various crude extracts of *T. officinale* have been reported, there is little report on the antimicrobial and antiproliferative activities of its solvent fractions.

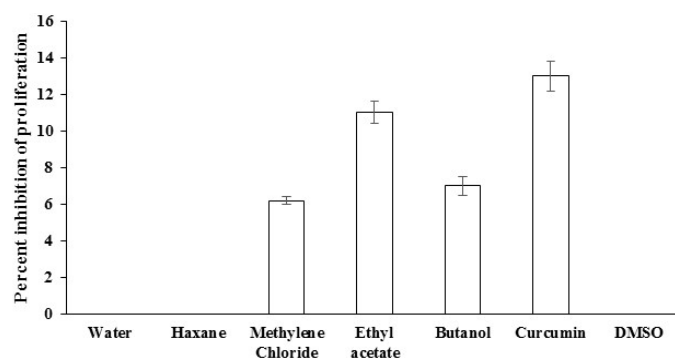
The aim of this study is therefore to provide a baseline data for the discovery of the active anticancer and antimicrobial phytochemicals in *T. officinale*. Crude methanolic extract of the dried leaves of *T. officinale* was fractionated in solvents of different polarities (hexane, methylene chloride, ethyl acetate, butanol and water) and the antiproliferative and antimicrobial activities of each fraction examined.

#### Antiproliferative activity and cytotoxicity

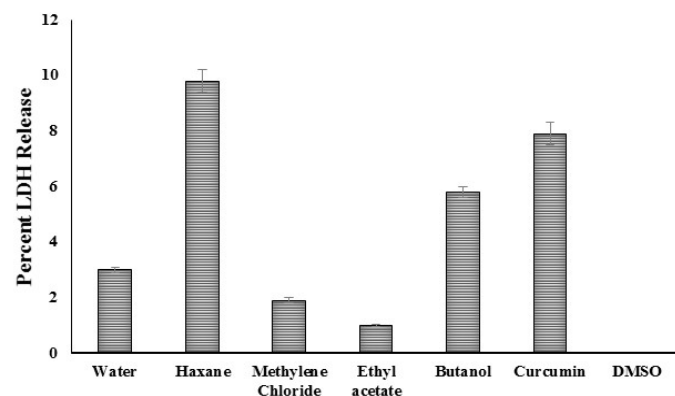
Figure 1 shows the effect of the 0.2 mg/ml of the solvent fractions of the crude methanolic extract of *T. officinale* on cancerous cells (SGT oral cancer cells) whilst figure 2 shows the effect on non-cancerous cells (RAW 264.7 macrophage cell). Intriguingly, the methylene chloride fraction demonstrated significant antiproliferative activity against the cancerous cells (Fig. 1) and not the non-cancerous cells (Fig. 2). The other fractions that showed significantly lower activity compared to the methylene chloride and curcumin also demonstrated selective activity against cancerous cells. The methylene chloride for instance inhibited as much as 97% of proliferation of the SGT cells (Fig. 1) and only about 7% of the RAW 246.7 cells (Fig. 2). Ethyl acetate and butanol fractions inhibited 42.03 % and 24.35 % proliferation of the SGT cells respectively and only 12% and 8% of the RAW 246.7 cells.



**Fig. 1:** Antiproliferative activity of extracts. The data represents the antiproliferative activity against SGT oral cancer cells by the extracts compared with curcumin. The results are mean values  $\pm$  S.E.M. of 3 independent experiments. \* Value significantly different ( $p < 0.05$ ) from the control group (Standard).



**Fig. 2:** Antiproliferative effects against RAW 264.7 macrophage cells. The results are mean values  $\pm$  S.E.M. of 3 independent experiments. \* Value significantly different ( $p < 0.05$ ) from the control group (Standard).



**Fig. 3:** Effects on cell membrane integrity compared to curcumin standard at 400  $\mu$ g/ml. The results are mean values  $\pm$  S.E.M. of 3 independent experiments. \*Value significantly different ( $p < 0.05$ ) from the control group (Standard).

This selective toxicity was also demonstrated by curcumin, a standard phenolic compound known for its effect on apoptosis of cancer cells (Andriana *et al.*, 2001). The selective toxicity demonstrated by the extracts is an important requirement of cancer chemotherapy (Chan and Gaccia, 2011). That is a good anticancer compound must selectively kill cancerous cells whilst sparing normal fast growing cells in the body. Many synthetic anticancer drugs however, have failed in this respect hence their numerous side effects (Chan and Gaccia, 2011).

**Table 1:** Total Phenolic and Flavonoid Content of Extracts.

Extract	Total Phenolic Content (mg gallic/g dry weight)	Total Flavonoid Content (mg quercetin/g dry weight)
Aqueous	1.5 ± 0.2	0.0 ± 0.0
Hexane	0.5 ± 0.0	1.0 ± 0.1
Methylene chloride	55.0 ± 2.0	4.4 ± 0.2
Ethyl acetate	34.0 ± 1.0	6.2 ± 0.4
Butanolic	5.0 ± 0.2	1.5 ± 0.1

Values are mean ± SEM. of 3 independent experiments.

**Table 2:** Antimicrobial activities of 50 mg/ml of solvent fractions of crude methanolic extract of *T. officinale* compared to 100 µg/ml of tetracycline by disc diffusion method.

Organism	Zone of inhibition (mm)			
	Butanol	Ethyl acetate	Methylene chloride	Tetracycline
<i>E. coli</i>	15.0 ± 0.5	15.0 ± 0.5	12.0 ± 0.5	18.0 ± 0.7
<i>S. aureus</i>	13.0 ± 0.0	18.0 ± 0.5	9.0 ± 1.0	17.0 ± 1.5
<i>B. subtilis</i>	19.0 ± 0.5	18.0 ± 0.5	11.0 ± 0.0	22.0 ± 0.7
<i>C. albicans</i>	20.0 ± 0.0	17.0 ± 1.0	14.0 ± 0.5	20.0 ± 0.6
<i>S. cerevisiae</i>	13.0 ± 0.0	15.0 ± 0.5	12.0 ± 0.5	18.0 ± 0.6

Results are means of diameters of zones of inhibition ± S.E.M for 3 independent assays.

**Table 3:** Percent growth inhibition of test organisms by 50 mg/ml solvent fractions of crude methanolic extract of *T. officinale* compared to 100 µg/ml tetracycline.

Organism	% Growth Inhibition				
	Butanol	Ethyl acetate	Methylene chloride	Aqueous	Tetracycline
<i>E. coli</i>	62.0 ± 4.0	18.0 ± 1.0	52.0 ± 2.0	14.0 ± 0.0	78.0 ± 4.0
<i>S. aureus</i>	76.0 ± 4.0	13.0 ± 0.0	49.0 ± 2.0	0.0 ± 0.0	75.0 ± 4.0
<i>B. subtilis</i>	54.0 ± 2.0	46.0 ± 2.0	18.0 ± 1.0	10.0 ± 0.0	84.0 ± 4.0
<i>C. albicans</i>	70.0 ± 4.0	64.0 ± 4.0	60.0 ± 4.0	0.0 ± 0.0	68.0 ± 4.0
<i>S. cerevisiae</i>	50.0 ± 0.4	50.0 ± 4.0	64.0 ± 4.0	0.0 ± 0.0	50.0 ± 2.0

Results are means ± S.E.M for 3 independent assays.

Furthermore, the fractions did not only showed selectivity but also demonstrated low cytotoxicity in the RAW microphage cells as determined by measuring the release of lactate dehydrogenase enzyme. The methylene chloride showed very low cytotoxic effect. At 0.4 mg/ml, none of the extracts showed a considerable cytotoxic effect via the release of LDH. The hexane fraction showed the highest toxicity (9.46 ± 0.59 %), followed by curcumin 7.29 ± 0.38 %. The percent LDH released by the butanol and water fractions were 5.04 ± 0.17 % and 2.45 ± 0.33 % respectively whereas the methylene chloride and ethyl acetate fractions released as low as 1.74 ± 0.11 % and 0.86 ± 0.02 % respectively (Fig. 3). The results in this study therefore indicate that the potential antiproliferative phytochemicals in *T. officinale* leaves as extracted in the methylene chloride fraction, could further explored to be developed into a safe anticancer agent.

The anticancer activity of medicinal plant extracts have been strongly linked to natural phenolic compounds (Andriana *et al.*, 2001). In the present study, the methylene chloride fraction has the highest phenolic content, containing as much as 55 GAE, followed by the ethyl acetate fraction (34 GAE) and then the butanol fraction (5 GAE) (Table 1). Thus, there is strong correlation between phenolic content and antiproliferative activity.

#### Antimicrobial activity

The antimicrobial activities against the various organisms used in this study as determined by the disc diffusion and broth dilution methods are presented in tables 2 and 3 respectively. With the exception of the hexane fraction,

which did not show any detectable inhibition (data not presented) all the other fractions showed broad antimicrobial activities inhibiting both gram-positive, gram-negative bacteria and the fungal species. The butanol fraction did not only showed very good broad activity but has shown a comparable activity to tetracycline. The aqueous fraction showed no activity in the disc diffusion test but showed moderate toxicity against *E. coli* and *B. subtilis* in the broth dilution test. The result in this study is consistent with those of Ghaima *et al.* (2013), who reported antimicrobial activity of crude extract of *T. officinale* against *S. aureus* and *E. coli*.

#### CONCLUSION

The results obtained in the present study provide an evidence that the phytochemicals responsible for antiproliferative and antimicrobial activities of *T. officinale* leaf extract have been extracted in the methylene chloride and butanolic fractions respectively. The potential antiproliferative phytochemicals have shown selectivity against cancer cell lines and very low cytotoxicity against non-cancerous cell lines. Thus, the methylene chloride and butanolic fractions can act as leads for the discovery of safe anticancer and antimicrobial agents from *T. officinale* leaves.

#### ACKNOWLEDGEMENT

The authors are grateful to Dongguk University, Gyeongju, South Korea and University of Ghana, Legon, Accra, Ghana for financial support.

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**How to cite this article:**

C.O. Tetty, A. Ocloo, P.C.N. Nagajyothi, and K.D. Lee., An *in vitro* analysis of antiproliferative and antimicrobial activities of solvent fractions of *Taraxacum officinale* (Dandelion) leaf. *J App Pharm Sci*, 2014; 4 (03): 041-045.