Phytochemical Profiling, Anticancer and Antimicrobial Activities of Ratanjot (*Alkanna tinctoria)* Root Extracts

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Abstract
A variety of plants are available by nature such as ornamental and medicinal. Medicinal plants contain many biologically active ingredients such as alkaloids, saponins, poly phenol, anthracyanin, terpenoids and flavonoids. These potent bio active components have therapeutic properties and have the ability to treat different health issues including stomach ulcers, inflammation, gastrointestinal issues, skin diseases, respiratory problems and various pathogenic diseases etc. Ratanjot (*Alkanna tinctoria)* is a herbaceous flowering plant. It contains many important phytochemicals and biological active compounds. It shows many pharmacological activities such as antioxidant, anti-proliferation, antimicrobial and anti-inflammatory. Ratanjot majorly contains two important naphthoquinone derivatives namely alkannins and shikonins. They are powerful components and show anti-inflammatory, antimicrobial and wound healing properties. The main objective of this study is to identify the important biological active natural compounds from the roots of *Alkanna tinctoria* by different extraction methods. Plant extracts will be prepared by the conventional and modern extraction methods. Phytochemical profiling will be carried out by UV-vis spectroscopy and Fourier transform infrared spectroscopy (FTIR). After characterization the extracts will be used to study the anti-cancer activity of hepatic carcinoma cell lines (Huh7 and HepG2) and will evaluate the anti-microbial activity. This study will be helpful for the treatment of liver cancer and infectious diseases. Antioxidant activity will also be performed for different roots extracts.

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Keywords: Phytochemical, Anticancer, *Alkanna tinctoria*, Antimicrobial

Introduction

A human relation with the plants is very old and is deeply rooted. Since the dawn of civilization human beings have depended upon the resources of the plants (Sandberg *et al*., 2003) [10]. Medicinal plants have become significant ever, as their role in preventing and treating a wide range of diseases is being more widely acknowledged (Rakotoarivelo *et al*., 2015) [7]. Such plants are the rich source of bioactive compounds that can be extracted, characterized and are used in the preparation of pharmaceutical products. People across the various ethnic and cultural groups still use raw and unprocessed plant material in order to cure diseases, especially in traditional medicine system (Yuan *et al*., 2016) [15]. The medicinal herbs can be attributed to their prospective healing qualities and have a wide range of secondary metabolites like flavonoids, tannins, alkaloids, and terpenoids. These natural products have been attributed to exhibit extensive pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial and anti-fungal (Talih *et al*., 2010) [14].

Traditional medicine has always used herbal plants due to their simple curative effects with less side effects. It is herbal medicines, as recent researches indicate, that have regained value as safe and sustainable sources in global healthcare systems

(Hilal *et al*., 2024) [4].

Herbal remedies have become vital because of their therapeutic nature. Therapeutic plants are used to treat a broad spectrum of diseases and health ailments such as: cancer, diabetes, cardiovascular diseases, immune deficiency, stroke, chronic obstructive pulmonary disease, high blood pressure, neurodegenerative disorders and infections (Souri *et al*., 2008) [11]. These plants are from different genera and families with a common usage as the extraction source of bioactive compounds or essential oils (Srinivasan *et al*., 2001) [12]. The biologically active compounds are found in different sections of the plants such as roots, stems, barks, leaves, flowers, fruits, and rhizomes. Isolates of these components have shown antioxidant, antibacterial and anti-fungal activities (Rahmatullah *et al*., 2009) [6].

Dyers bugloss, also called alkanet, is a source of red dye. *Alkanna tinctoria* belongs to borage family which is called Boraginaceae. It has very striking blue flowers and is a perennial herb. It in general grows to a height of approximately 1520 cm and a diameter of approximately 3 cm. It is hermaphroditic making it have both male and female reproductive organs. Arabic name of this plant al-hena has a Spanish origin, the term alcana is connected with henna (Lawsonia inermis). Alkanet is also known by many other names such as Ratanjot root, orchanet, Spanish bugloss and Languedoc bugloss. Its flowers are in full blossom in the summer seasons (May-July). The outer part of the root is dark reddish-black, and the inside of the root is reddish red and white at the center. The foliage of the plant is greyish-green and covered with a combination of short and stiff hair some bear little bumps, and non-glandular hair (Inoue *et al*., 2019) [5].

Research Objectives:

1. To perform phytochemical profiling of *Alkanna tinctoria* root extract using phytochemical screening.
2. To evaluate them *in vitro* anticancer and antimicrobial activities.

Research Questions:

1. What are the major phytochemical constituents of *Alkanna tinctoria* root extracts?
2. How the constituents related to *Alkanna tinctoria in vitro* anticancer and antimicrobial activities?

Literature Review

Takc *et al.,* (2019) determined the safety of *Alkanna tinctoria* root extracts. The work showed the nature of these extracts in inhibiting harmful bacteria and fungi and test their toxicity to healthy cells. Simple methods employed were solvent technique in extracting the roots, agar well diffusion to determine the antimicrobial activity, and broth dilution to quantify the antimicrobial activity and MTT determination of the antimicrobial activity of cytotoxicity against the normal cells. The findings depicted the root extracts as successful in inhibiting growth of a number of bacteria and fungi species, which is a very powerful antimicrobial potential. The extracts were toxic to healthy cells to a low extent showing that they can be used safely in the condition of any therapeutic purposes. The study justifies the application of *Alkanna tinctoria* root extracts as natural antimicrobial agents having minimum side effects.

Alwahibi & Perveen, (2017) [2]. investigated the chemical constituent and antimicrobial activity of *Alkanna tinctoria* root extract on skin infection causing bacteria. Water and ethanol extracts were prepared and used on common skin pathogens. The ethanol extract was highly effective as an antibacterial agent in particular against such bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* whereas the water extract was not so effective. The ethanol extract showed the presence of several natural compounds, among them naphthoquinones and esters that have medicinal value, as determined by GC-MS analysis. This finding concurs with the established tradition of using Alkanna tinctoria roots as an anti-infectious agent against skin infections and indicates that the extracts of the plant may be further investigated to have a potential of developing natural antibacterial sources.

Rani *et al.,* (2023) [8]. tested the ability of *Alkanna tinctoria* to act against glioma cells as an anti-cancer agent. To prepare the plant extract, the extraction was done with methanol at room temperature. After extraction it was filtered and concentrated. Extract concentrations were applied to the glioma cells and allowed to incubate between 24 and 48 hours. They determined cytotoxicity by MTT assay, which is a method of measuring cell survival through mitochondrial activity. Flow cytometry was assessed to determine early and late stages of cell death by staining with annexin V and propidium iodide. The parameters such as temperature (37 ^{o}C) and incubation times were carefully controlled in the study so as to be accurate. The extract was found to reduce cell survival and amplify the apoptotic effect, which implies the use of *Alkanna tinctoria* as a natural anti-cancer agent.

Materials and Methods

The research work was done as an experimental work at the Institute of Chemistry, University of Sargodha, Pakistan. In June 2025, *Alkanna tinctoria* was purchased at a local market in Sargodha, Pakistan. The roots of *Alkanna tinctoria* were washed using tap water and were then dried at room temperature (37 ^{o}C) in a shaded place for one week. The dried roots were ground into a fine powder. The powder was placed in a container. 70% ethanol was used to prepare the extract by two different methods

1. Conventional method (Maceration)
2. Modern method (Sonication)

Conventional Method (Maceration)

15g of root powder of plant was weighed using analytical balance. 100 ml of 70% ethanol was mixed with the plant powder. The conical flask containing the mixture was placed on shaker (OS-752, Tokyo, Japan) at a speed of 180 rpm (rounds per minute) over a period of 24 hours.

Modern Method (Sonication)

15 g of plant root powder was mixed with 100 ml of 70% ethanol in a beaker and sonicated at 25 ^{o}C for 20 minutes. Whatman No. 1 filter paper was used to filter the extracted solution. The filtrate was further evaporated at 50 ^{0}C in a water bath.

Antibacterial Activity

Antibacterial action of the extracts was determined by the disc diffusion technique. Glasses and Petri plates together with paper discs were initially sterilized within an autoclave. A sterilized LB agar (20-25 mL) was added to each Petri plate and left to cool down to 70 ^{o}C. Approximately 20-24 uL of the extract was put into each disc. It was then incubated at 37

°C for 24 hours on the Petri plates. The discs were incubated and then the clear areas around the discs were measured using a ruler in millimeters. A disc with no extract was taken as a negative control whereas there was 30 µg Moxiget on a disc which served as positive control. The diameter of zones of inhibition of the extracts obtained were compared to the control samples in millimeters.

Anticancer Activity

Multi-well plates were seeded with cancer cells and left to grow and attach within a given time. This was followed by treatment of the cells with various concentrations of the root extracts. The unstimulated cells were used as a control group, and a known anticancer drug could be applied as a positive control. The control drug was Doxorubicin. The treated cells were then incubated to have a fixed time to enable the extract to interact with the cells.

MTT (Methyl Thiazolyl Tetrazolium Assay) tetrazolium reagent was placed in every well following the treatment period. Living cells converted this reagent into a colored reagent whereas dead cells did not. The degree of the formed color is directly proportional to the quantity of viable cells. Incubation was then followed by dissolution of the color in an appropriate solvent followed by measurement of the absorbance in a microplate reader.

Characterizations of Alkanna tinctoria root extracts

Ultraviolet-visible (UV-VIS) Spectroscopic analysis

UV-visible spectrophotometric analysis of the extract of the *Alkanna tinctoria* was carried out at room temperature using the UV-VIS spectrophotometer. Both the ultraviolet and visible light were used to scan the extract at a wavelength of 300nm to 800 nm. The extract was centrifuged at 3000 rpm for 10 minutes to remove any solid particles before analysis with Whatman no. 1 filter paper. A 1:10 ratio was used to dilute the filtered sample with the same solvent to get the appropriate concentration to be analyzed. The absorbance values were recorded and the highest UV-VIS peaks were recorded to be further interpreted.

Fourier-transform infrared (FTIR) Spectroscopic analysis

Alkanna tinctoria extract was combined with a little amount of potassium bromide (KBr). The blend was well crushed in a mortar to form a fine and homogenous powder. The powder was then mixed properly and the mixture was pressed under a pressure of 6 bars in the order of two minutes to create a thin KBr disc. The prepared disc was introduced in the sample cup of a diffuse reflectance accessory. The recorded infrared spectrum was at Bruker Vertex 70 FTIR spectrometer manufactured in Germany. The frequency used to scan the sample ranged between 4000 and 400 cm^{-1}. The spectrum was analyzed to identify specific absorption bands of various functional groups that were in the extract.

Results and Discussions

Antibacterial Activity

The activity was checked against *Bacillus subtilis* and *Escherichia coli.* The extract prepared by maceration showed a visible antibacterial effect in all the microorganisms tested. It gave a zone of inhibition of 17 mm against *B. subtilis* and 19 mm against *E. coli.* The extract prepared by sonication showed the zone of inhibition 25 mm against *B. subtilis* and 17 mm against *E. coli.* Moxiget was used as a standard drug. It gave zone of inhibition of 30 mm against *B. subtilis* and 25 mm against *E. coli.* A study by Das *et al*., (2024) [3] confirmed the antimicrobial activity of *Alkanna tinctoria* root extract against *Listeria monocytogenes, Staphylococcus aureus and Salmonella enterica.*

Table 1: Zone of inhibition against bacterial strains for Alkanna tinctorial

Code	Zone of Inhibition (mm)		
	Extracts of *Alkanna tinctoria*	Gram +ve Bacteria	Gram –ve Bacteria
		Bacillus subtilis (Hay bacillus)	*Escherichia coli*
1	Maceration	17	19
2	Sonication	25	17
3	Standard Drug	30 (Moxiget)	25 (Moxiget)

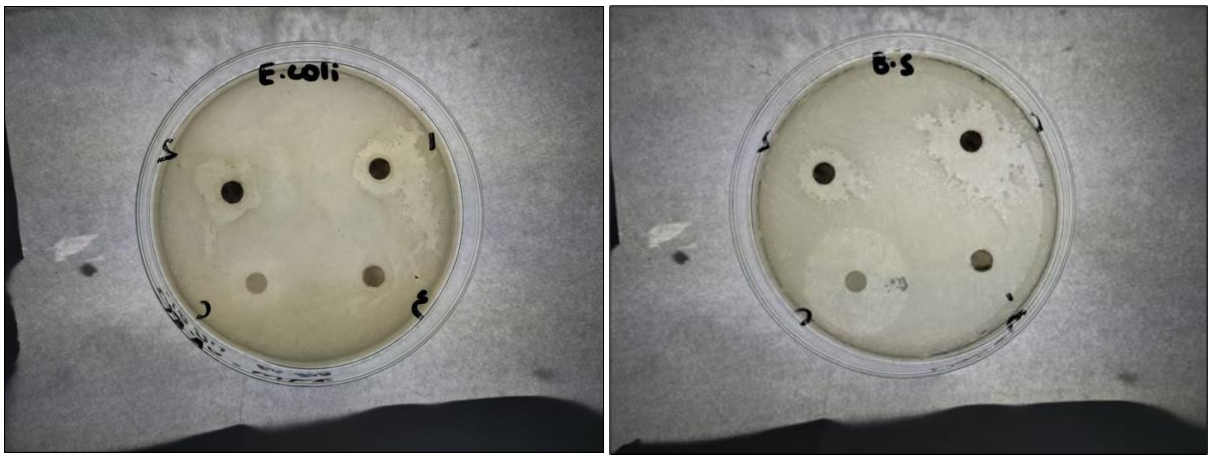

Fig 1: Antibacterial activity of *Alkanna tinctoria* root extracts

Anticancer Activity

The extracts showed that cell viability of both of the tested cancer cells declined when exposed to higher concentrations. The anticancer activity of sonication extract was the strongest against Huh7 cells (IC_{50}: 2.98 µg/mL, IC_{70}:4.89 µg/mL), followed by the HepG2 cells (IC_{50}:3.76 µg/mL, IC_{70}:5.56 µg/mL). The maceration extract exhibited a relatively weaker anticancer activity against the Huh7 (IC_{50}: 3.98 µg/mL, IC_{70}:5.55 µg/mL) and HepG2 (IC_{50}: 4.65 µg/mL, IC_{70}:6.34 µg/mL) cells.

Alotaibi *et al* (2024) [1]. studied the anticancer activity of nanoparticles of Alkanna tinctoria on HepG2 cancer lines. It confirmed the results shown in the present work. Rashan *et al* (2018) [9]. showed that A. tinctoria root extracts also had a high level of anti-proliferation activity against a panel of human tumor cell lines and as such, have the potential to act as natural anticancer agents.

Table 2: Anticancer Activity of *Alkanna tinctoria* root extract

***Alkanna tinctoria* ethanolic root extract**	**Cancer Cell Lines**			
	Huh7		**HepG2**	
	IC_{50} µg/mL	**IC_{70} µg/mL**	**IC_{50} µg/mL**	**IC_{70} µg/mL**
Maceration	3.98	5.55	4.65	6.34
Sonication	2.98	4.89	3.76	5.56

Characterization of Root extract FTIR analysis

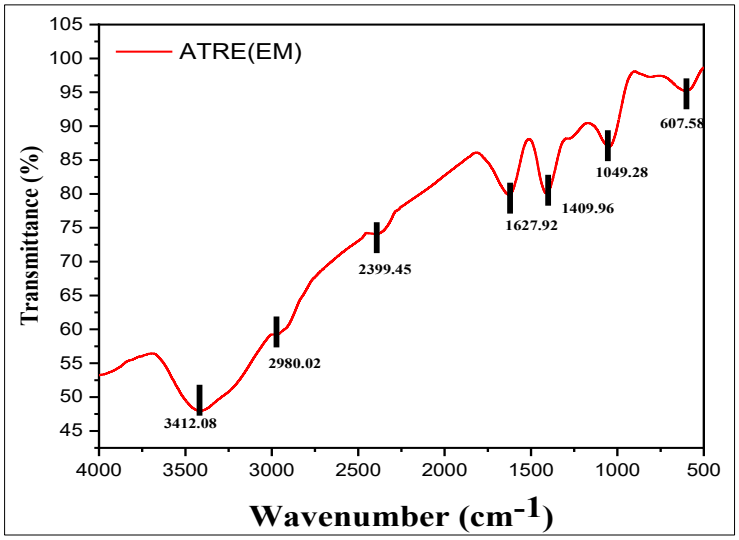

Fig 2: FTIR analysis of extract prepared by maceration

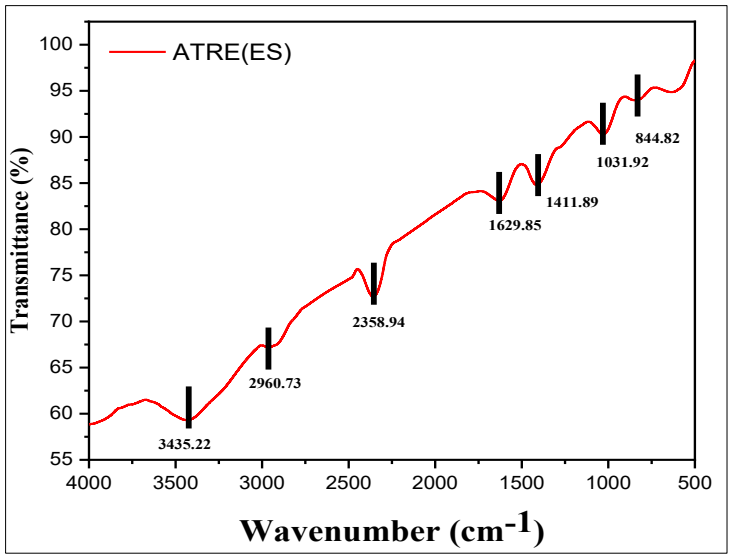

Fig 3: FTIR analysis of extract prepared by sonication

UV-Vis analysis

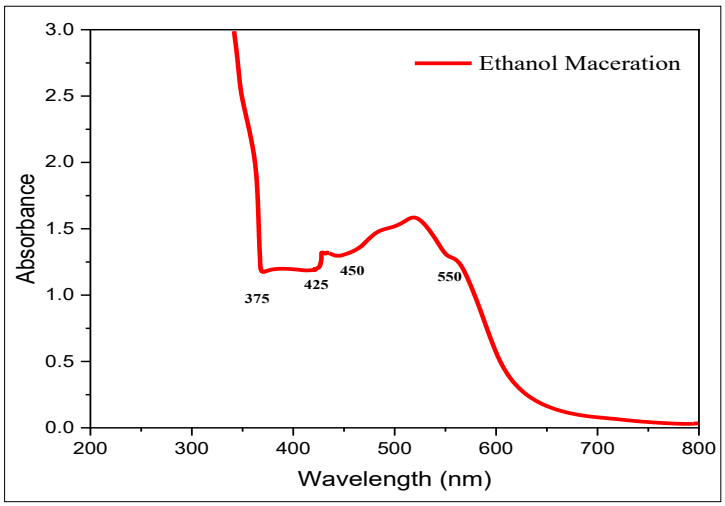

Fig 4: UV-vis analysis of extract by maceration

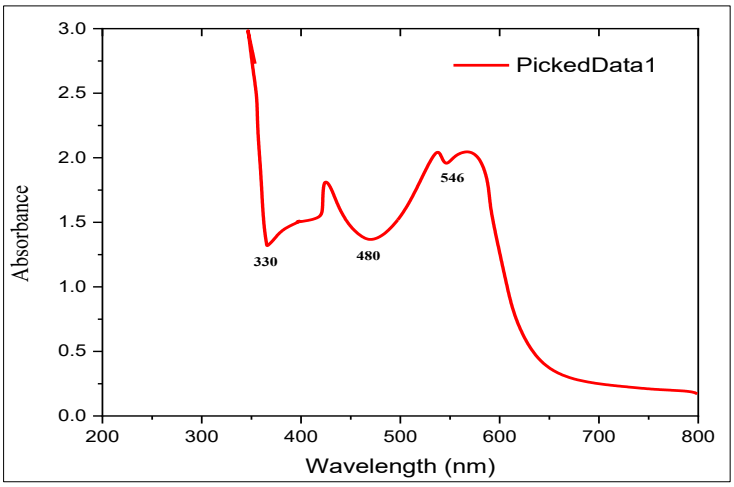

Fig 5: UV-vis spectroscopy of extract by sonication

Conclusion

The findings of this research indicated that all the two extracts that were produced out of roots of Alkanna tinctoria exhibited an observable antibacterial and anticancer activity. Phytochemical screening showed that the roots have a number of important bioactive products namely tannins, alkaloids, flavonoids and other phenolic compounds. These biologically active compounds are capable of showing biological activities. Experiments of the antibacterial effects of the extracts were conducted against two bacterial strains in the disc diffusion technique. The results indicated the use of the inhibitory action against Gram-positive and Gram-negative bacteria. Surprisingly, the greatest areas of inhibition were recorded with the extract prepared by sonication method. It portrays a potentially intense effect on antibacterial activity. Similarly, the anticancer activity was recorded on the two cancer cell lines namely Huh7 and HepG2. The extract prepared by sonication method showed greater anticancer activity than the extract prepared by maceration. All in all, the joint chemical and biological experiments indicate that the roots of this plant could have a potential in the field of therapeutic use.

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